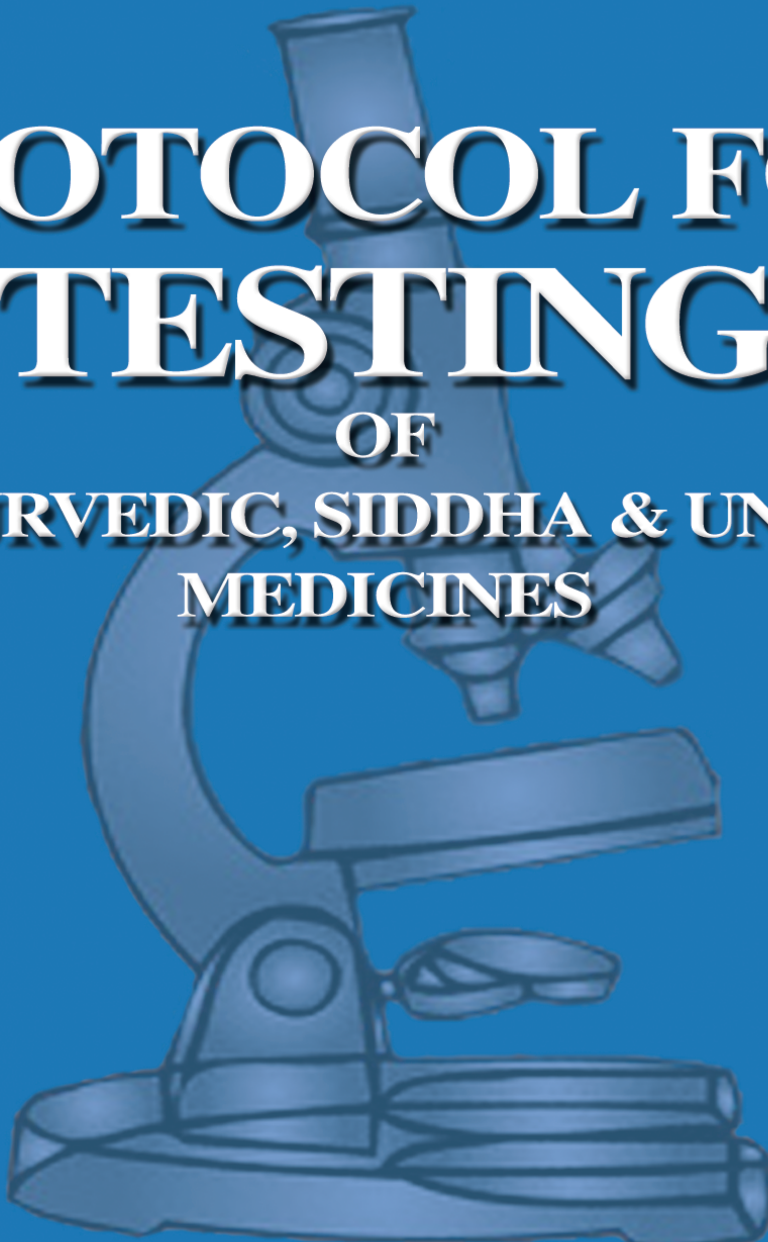
PROTOCOL FOR TESTING

OF

AYURVEDIC, SIDDHA & UNANI MEDICINES



सत्यमेव जयते

Government of India
Department of AYUSH
Ministry of Health & Family Welfare
PHARMACOPOEIAL LABORATORY FOR INDIAN MEDICINES
GHAZIABAD

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PREFACE

With the growing awareness of health care and safety aspects, people are moving towards Herbal products. In last a few decades, market of herbal and traditional medicines have grown up leap and bound. China is a major producer and exporter of Herbal and traditional medicine in the world and India has a very little share in this field. India can have a big role to play in this field as Indian system of medicine, Ayurveda and Siddha are thousands years old and time tested. Similarly Unani is also an ancient system of medicine, migrated from Iran, Persia and Gulf countries to India.

Indian herbal and traditional medicines Industries have annual turnover of about Rs.4,000-6,000 crore market and have big export potential. For export to western countries, America and Canada, there strict quality parameters and quality control and safety and efficacy are required. On this front our industries are not able to produce documentary support. Govt. of India and export dealing agency; PHARMEXIL are very much concern to promote export. For this, Export Inspection Council is also doing effort to lay down limits for safety parameters. It is also felt that protocol for testing of Ayurveda, Siddha and Unani products are not available and without which industries and various Govt. laboratories are not able to concentrate more on this issue. Then Department of AYUSH assigned Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad to develop protocol for testing. Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad prepared the draft document, sent to various experts of the field and after consideration of the comments received from these experts, final document has been prepared.

The present volume of protocol for testing of Ayurveda, Siddha and Unani product contains various parameters for testing for different categories of single and compound formulations, limits for heavy metals, microbial load, pesticide residue and aflatoxins, methods for determination of these safety and quality parameters. These methods have been taken from different official Pharmacopoeia like API, IP, BP, USP, EP and Chinese Pharmacopoeia.

These methods are well proved. These methods will also be made official by including in coming volumes of API. Though every care has been taken to prepare this document, however if there is any controversy, official methods and official parameters and the limit given in official monographs of pharmacopoeia will be final.

Dr. D.R. Lohar

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Dr. D.R. Lohar

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GENERAL NOTICES

Name of the Drugs - The name given on the top of each monograph of the drug is in Sanskrit as mentioned in the Ayurvedic classics and/or in the Ayurvedic Formulary of India, Part-I and Part-II will be considered official. These names have been arranged in English alphabetical order. The Latin name (taxonomical nomenclature) of each drug as found in authentic scientific literature has been provided in the monograph in the introductory paragraph. The official name will be the main title of the drug and its scientific name will also be considered as legal name.

Italics - Italic type has been used for scientific name of the drug appearing in the introductory paragraph of each monograph as also for chemicals and reagents, substances or processes described in Appendix.

Odour and Taste - Wherever a specific odour has been found it has been mentioned but the description as 'odourless' or 'no odour' has in many cases been avoided in the description, as large numbers of drugs have got no specific odour. The "odour" is examined by directly smelling 25 g of the powdered drug contained in a package or freshly powdered. If the odour is discernible the sample is rapidly transferred to an open container and re-examined after 15 minutes. If the odour persists to be discernible, it is described as having odour.

The "Taste" of a drug is examined by taking a small quantity of 85 mesh powder by a tip of moist glass rod and applying it on tongue previously rinsed with water. This may not be done in case if poisonous drugs, indicated in monograph.

Mesh Number - Wherever the powdering of the drug has been required the sieve "Mesh Number 85" has been used. This will not apply for drugs containing much oily substance.

Weights and Measures - The metric system of weights and measures is employed. Weights are given in multiples or fractions of a gramme (g) or of a milligram (mg). Fluid measures are given in multiples or fractions of millilitre (ml).

When the term "drop" is used, the measurement is to be made by means of a tube, which delivers in 20 drops 1 gram of distilled water at 15^{o}C.

Metric measures are required by the Pharmacopoeia to be graduated at 20^{o}C and all measurements involved in the analytical operations of the Pharmacopoeia are intended, unless otherwise stated to be made at that temperature.

Identity, Purity and Strength - Under the heading "Identification" tests are provided as an aid to identification and are described in their respective monographs.

Foreign Matter - The term is used to designate any matter, which does not form part of the drug as defined in the monograph. Vegetable drugs used as such or in formulations, should be duly identified and authenticated and be free from insects, pests, fungi, micro-organisms, pesticides, and other animal matter including animal excreta, be within

the permitted and specified limits for lead, arsenic and heavy metals, and show no abnormal odour, colour, sliminess, mould or other evidence of deterioration.

The quantitative tests e.g. total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, water- soluble extractive, ether-soluble extractive, moisture content, volatile oil content and assays are the methods upon which the standards of Pharmacopoeia depend. The methods for assays are described in their respective monographs and for other quantitative tests, methods are not repeated in the text of monographs but only the corresponding reference of appropriate appendix is given. The analyst is not precluded from employing an alternate method in any instance if he is satisfied that the method, which he uses, will give the same result as the Pharmacopoeial Method. In suitable instances the methods of microanalysis, if of equivalent accuracy, may be substituted for the tests and assays described. However, in the event of doubt or dispute the methods of analysis of the Pharmacopoeia are alone authoritative.

Standards - For statutory purpose, statements appearing in the API, Part-I, Vol. I to V under Description, those of definition of the part and source plants, and Identity, Purity and Strength, shall constitute standards.

Thin Layer Chromatography (T.L.C.) - Under this head, wherever given, the number of spots and Rf values of the spots with their colour have been mentioned as a guide for identification of the drug and not as Pharmacopoeial requirement. However, the analyst may use any other solvent system and detecting reagent in any instance if he is satisfied that the method which he uses, even by applying known reference standards, will give better result to establish the identity of any particular chemical constituent reported to be present in the drug.

Quantities to be weighed for Assays and Tests - In all description quantity of the substance to be taken for testing is indicated. The amount stated is approximate but the quantity actually used must be accurately weighed and must not deviate by more than 10 per cent from the one stated.

Constant Weight - the term "Constant Weight" when it refers to drying or ignition means that two consecutive weighings do not differ by more than 1.0 mg per g of the substance taken for the determination, the second weighing following an additional hour of drying on further ignition.

Constituents - Under this head only the names of important chemical constituents, groups of constituents reported in research publications have been mentioned as a guide and not as pharmacopoeial requirement.

Percentage of Solutions - In defining standards, the expression per cent (%), is used, according to circumstances, with one of the four meanings given below.

Per cent w/w (percentage weight in weight) expresses the number of grammes of active substance, in 100 grammes of product.

Per cent w/v (Percentage weight in volume) expresses the number of grammes of active substance in 100 millilitres of product.

Per cent v/v (percentage volume in volume) expresses the number of millilitres of active substance in 100 millilitres of product.

Per cent v/w (percentage volume in weight) expresses the number of millilitres of active substance in 100 grammes of product.

Percentage of alcohol - All statements of percentage of alcohol (C_2H_5OH) refer to percentage by volume at 15.56 ^{0}C.

Temperature - Unless otherwise specified all temperatures refer to centigrade (celsius), thermometric scale.

Solutions - Unless otherwise specified in the individual monograph, all solutions are prepared with purified water.

Reagents and Solutions - The chemicals and reagents required for the test in Pharmacopoeia are described in Appendices.

Solubility - When stating the solubilities of Chemical substances the term "Soluble" is necessarily sometimes used in a general sense irrespective of concomitant chemical changes.

Statements of solubilities, which are expressed as a precise relation of weights of dissolved substance of volume of solvent, at a stated temperature, are intended to apply at that temperature. Statements of approximate solubilities for which no figures are given, are intended to apply at ordinary room temperature.

When the expression "parts" is used in defining the solubility of a substance, it is to be understood to mean that 1 gramme of a solid or 1 millilitre of a liquid is soluble in that number of millilitres of the solvent represented by the stated number of parts.

When the exact solubility of pharmacopoeial substance is not known, a descriptive term is used to indicate its solubility.

The following table indicates the meaning of such terms :-

Descriptive terms	*Relative quantities of solvent*
Very soluble	Less than 1 part.
Freely soluble	From 1 to 10 parts.
Soluble	From 10 to 30 parts.
Sparingly soluble	From 30 to 100 parts.
Slightly soluble	From 100 to 1000 parts.
Very slightly soluble	From 1000 to 10,000 parts.
Practically insoluble	More than 10,000 parts.

Abbreviations of technical terms – The abbreviations commonly employed are as follows :

m	Metre
l	Litre
mm.	Millimetre
cm.	Centimetre
μ	Micron (0.001 mm)
Kg.	Kilogram
g.	Gramme
mg.	Milligram
ml.	Millilitre
IN.	Normal solution
1M.	Molar solution
Fam.	Family
PS.	Primary Standards
TS.	Transverse Section

ANALYTICAL SPECIFICATIONS OF ARKA/THEENEER/DISTILLATES

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	pH
3.	Volatile matter
4.	Specific gravity at 25^{0} C
5.	Clarity test
6.	Identifications, TLC/HPTLC/GLC
7.	Assay
8.	Test for heavy metals Lead Cadmium Mercury Arsenic
9.	Microbial contamination Total bacterial count Total fungal count
10.	Test for specific Pathogen E. coli *Salmonella spp.* *S. aureus* Pseudomonas aeruginosa
11.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids

ANALYTICAL SPECIFICATIONS OF ASAVA AND ARISHTA (FERMENTED LIQUIDS)

Sl. No.	Tests
1.	Description
	Colour
	Odour
2.	pH
3.	Specific gravity at 25^0 C
4.	Total solids
5.	Alcohol content Test for methanol
6.	Reducing sugar
7.	Non-reducing sugar
8.	Identifications, TLC/HPTLC
9.	Total acidity
10.	Test for heavy metals Lead Cadmium Mercury Arsenic
11.	Microbial contamination Total bacterial count Total fungal count
12.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
13.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids

ANALYTICAL SPECIFICATIONS OF AVACHURNAM YOGA (AYURVEDIC DUSTING POWDER)

Sl.No.	Tests	
1.	Description	
	Colour	
	Odour	
2.	Identification	
	Microscopic	
	Identifications, TLC/HPTLC	
3.	Particle size	mesh size 125-150
4.	Total – ash	
5.	Acid – insoluble ash	
6.	Water – soluble extractive	
7.	Alcohol – soluble extractive	
8.	Loss on drying at 105^{0}C	
9.	Test for metals Magnesium, Silica, Chloride	 Carbonate AluminiumIron,
10.	Test for heavy metals Lead Cadmium Mercury Arsenic	
11.	Microbial contamination Total bacterial count Total fungal count	
12.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa	
13.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids	
14	Test for Aflatoxins (B_1,B_2,G_1,G_2)	

ANALYTICAL SPECIFICATIONS OF AVALEHA /LEHAM/ ILAGAM (CONFECTIONS /SEMI SOLID)

Sl.No.	Tests
1.	Description
	Colour
	Odour
	Taste
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	pH
6.	Total solid
7.	Fat content
8.	Reducing sugar
9.	Total sugar
10.	Identifications, TLC/HPTLC
11.	Test for heavy metals Lead Cadmium Mercury Arsenic
12.	Microbial contamination Total bacterial count Total fungal count
13.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
14.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
15	Test for Aflatoxins (B_1,B_2,G_1,G_2)

ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM (FINE POWDER)/KVATHA CURNA/KUTINIR CHOORNAM (COARSE POWDER FOR DECOCTION)

Sl.No.	Tests
1.	Description
	Macroscopic
	Microscopic
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for *Churna;* 40-60 mesh for *Kvatha churna*)
8.	Identifications, TLC/HPTLC-with marker (wherever possible)
9.	Test for heavy/Toxic metals Lead Cadmium Mercury Arsenic
10.	Microbial contamination Total bacterial count Total fungal count
11.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
12.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
13	Test for Aflatoxins (B_1,B_2,G_1,G_2)

ANALYTICAL SPECIFICATIONS OF LEPA/MALHARA/KALIMBU/PASAI (MEDICATED WAX/CREAM/POULTICE)

Sl.No.	Tests
1.	Description
	Colour
	Odour
	Consistency/Uniformity of content
	Microscopic (if powdered drugs incorporated)
	Rancidity test
	Identifications, TLC /HPLC/GLC
	Assay (Wherever possible)
2.	Viscosity
3.	pH
4.	Particle size (if powdered drugs incorporated) mesh size 125-150
5.	Total fatty matter
6.	Loss on drying at 105^0 C
7.	Spreadability
8.	Test for heavy metals Lead Cadmium Mercury Arsenic
9.	Microbial contamination Total bacterial count Total fungal count
10.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
11.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids

ANALYTICAL SPECIFICATIONS OF, NETRA BINDU, ANJANA/KARN BINDU, CHOTTU MARUNTHU/(EYE DROPS)

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	pH
3.	Clarity test
4.	Sterility test
5.	Identifications TLC/HPTLC/GLC
6.	Assay

It should complies to Schedule FF of Drugs & cosmetics Act for concentration of preservatives and other requirements.

ANALYTICAL SPECIFICATIONS OF VARTTI

Sl.No.	Tests
1.	Description
	Colour
	Odour
	Uniformity of content
2.	Hardness (wherever applicable)
3.	Melting temperature
4.	Identifications TLC/HPTLC/HPLC
5.	Loss on drying at 105^0 C
6.	Water-soluble extractive
7.	Alcohol – soluble extractive
8.	Volatile oil
9.	Assay (if possible)
10.	Test for heavy/Toxic metals Lead Cadmium Mercury Arsenic
11.	Microbial contamination Total bacterial count Total fungal count
12.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
13.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids

ANALYTICAL SPECIFICATIONS OF PISHTI/CHUNNAM (PROCESSED FINE POWDER)

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	Taste
3.	Identification
4.	Assay of element (s)
5.	Loss on drying at 105 °C
6.	Total-ash
7.	Acid – insoluble ash
8.	Particle size mesh size 125-150
9.	Test for heavy/Toxic metals Lead Cadmium Mercury Arsenic
10.	Microbial contamination Total bacterial count Total fungal count
11.	Test for specific Pathogen E. coli Salmonella spp. S.aureus Pseudomonas aeruginosa
12.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
13	Test for Aflatoxins (B_1,B_2,G_1,G_2)

ANALYTICAL SPECIFICATIONS OF GHANSATVA/PLANT EXTRACTS

Sl.No.	Tests
1.	Description
	Colour
	Odour
	Taste
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	pH
6.	Water-soluble extractive
7.	Alcohol – soluble extractive
8.	Identifications TLC/HPTLC/HPTLC-Profile with marker
9.	Test for heavy/Toxic metals Lead Cadmium Mercury Arsenic
10.	Microbial contamination Total bacterial count Total fungal count
11.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
12.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
13.	Test for Aflatoxins (B_1,B_2,G_1,G_2)

ANALYTICAL SPECIFICATIONS OF GHRTA AND TAILA (MEDICATED OIL AND GHEE)

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	Weight/ml. (in case of taila)
3.	Refractive index at 25 ºC
4.	Viscosity
5.	Iodine value
6.	Saponification value
7.	Acid value
8.	Peroxide value
9.	Identifications GLC/TLC/HPTLC – with marker wherever available
10.	Test for heavy metals Lead Cadmium Mercury Arsenic
11.	Microbial contamination Total bacterial count Total fungal count
12.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
13.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
14	Test for Aflatoxins (B1,B2,G1,G2)

ANALYTICAL SPECIFICATIONS OF GUGGULU

Sl.No.	Tests
1.	Description
	Colour
	Odour
	Taste
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	pH
6.	Identification TLC/HPTLC/HPTLC-Profile with marker
7.	Water-soluble extractive
8.	Alcohol – soluble extractive
9.	Test for heavy / Toxic metals Lead Cadmium Mercury Arsenic
10.	Microbial contamination Total bacterial count Total fungal count
11.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
12.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
14.	Test for Aflatoxins (B1,B2,G1,G2)

ANALYTICAL SPECIFICATIONS OF VATI/GUTIKA/MODAKA/KULIGAI/ MARTHIRAI/VADAGAM (TABLET/PILLS)

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	Weight variation
3.	Disintegration time Not more than 15 minutes Not more than 60 minutes – guggulu tablets
4.	Identification TLC/HPTLC/GLC
5.	Assay
6.	Test for heavy/Toxic metals Lead Cadmium Mercury Arsenic
7.	Microbial contamination Total bacterial count Total fungal count
8.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
9.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
11	Test for Aflatoxins (B_1,B_2,G_1,G_2)

ANALYTICAL SPECIFICATIONS OF SYRUP, SHAARKAR/SHARBAT/MANAPPAGU

Sl.No.	Tests
1.	Description, Colour
2.	Odour
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	PH
8.	Total sugar content
9.	Viscosity
10.	Identification TLC/HPTLC/HPLC
11.	Test for heavy metals Lead Cadmium Mercury Arsenic
12.	Microbial contamination Total bacterial count Total fungal count
13.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* Pseudomonas aeruginosa
14.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids

ANALYTICAL SPECIFICATIONS OF BHASMA/SINDURA/ PARPAM/CHENDURAM (CALX)

Sl.No.	3. Tests
1	Description
	Colour
	Odour
2	Identification -chemical
3	Particle size mesh size — 200 - 300
4	Loss on drying at 105^0 C
5	Total – ash
6	Acid – insoluble ash
7	Water soluble ash
8	Assay of element (s)
9	Ayurvedic specifications
10	Lustreless (*Nishchandrica)*
11	Fine enough to enter the crevices of finger (*Rekha purnatva)*
12	Floats on water (*Varitara*)
13	Smokeless (*Nirdhoom*)
14	Tasteless (*Niswadu*)
15	Irreversible (*Apunar bhav*)

ANALYTICAL SPECIFICATIONS OF MANDURA

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	Identification -Chemical
3.	Particle size mesh size 200—300
4.	Loss on drying at 105^0 C
5.	Total – ash
6.	Acid – insoluble ash
7.	Water soluble ash
8.	Assay of element (s)
9.	Ayurvedic specifications
10.	Lustreless (*Nishchandrica)*
11.	Fine enough to enter the crevices of finger (*Rekha purnatva)*
12.	Floats on water (*Varitara*)
13.	Smokeless (*Nirdhoom*)
14.	Tasteless (*Niswadu*)
15.	Irreversible (*Apunar bhav*)

ANALYTICAL SPECIFICATIONS OF RASAYOGA

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	Identification –Chemical
3.	Particle size mesh size 200—300
4.	Loss on drying at 105^0 C
5.	Total – ash
6.	Acid – insoluble ash
7.	Water soluble ash
8.	Assay of element (s)
9.	Ayurvedic specifications
10	Lustreless (Nishchandrica)
11	Fine enough to enter the crevices of finger (Rekha purnatva)
12	Floats on water (Varitara)
13	Smokeless (Nirdhoom)
14	Tasteless (Niswadu)
15	Irreversible (Apunar bhav)

ANALYTICAL SPECIFICATIONS OF LAUHA

Sl.No.	Tests
1.	Description
	Colour
	Odour
2.	Identification
3.	Particle size mesh size 200-300
4.	Loss on drying at 105^0 C
5.	Total – ash
6.	Acid – insoluble ash
7.	Water soluble ash
8.	Assay of element (s)
9.	Ayurvedic specifications
10	Lustreless (*Nishchandrica)*
11	Fine enough to enter the crevices of finger (*Rekha purnatva)*
12	Floats on water (*Varitara*)
13	Smokeless (*Nirdhoom*)
14	Tasteless (*Niswadu*)
15	Irreversible (*Apunar bhav*)

ANALYTICAL SPECIFICATIONS OF SINGLE PLANT MATERIAL

Sl.No.	Tests
1.	Identification
	Macroscopic
	Microscopic
	Powder characteristics
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	TLC/HPTLC-Profile with marker (wherever possible)
6.	Water-soluble extractive
7.	Alcohol – soluble extractive
8.	Assay
9.	Test for heavy/Toxic metals Mercury Lead Cadmium Arsenic
10.	Microbial contamination Total bacterial count Total fungal count
11.	Test for specific Pathogen E. coli *Salmonella spp.* *S.aureus* *Pseudomonas aeruginosa*
12.	Pesticide residue Organochlorine pesticides Organophosphorus pesticides Pyrethroids
13.	Test for Aflatoxins (B_1,B_2,G_1,G_2)

LIMITS FOR ASU PRODUCTS

Parameter	Specifications
Total Bacterial count	1 x 10^5 CFU/gm
Yeast & Mould	1 x 10^3 CFU/gm
E. coli	Absent
. Salminella	Absent
P. aeruginosa	Absent
S. aureus	Absent
2. Pesticide Residue – Organo-chloro group **	Less than 1 ppm
3. Heavy metals	
Lead	10 ppm
Mercury	01 ppm
Arsenic	03 ppm
Cadmium	0.3 ppm
4. Aflatoxin	B1 - 0.5 ppm G1 - 0.5 ppm B2 - 0.1 ppm G2 - 0.1 ppm

**In the specifications for Pesticide residue it was decided to allow a maximum of 1 ppm for Organo Chloro group. In case there is a failure of the same then the 7 pesticides mentioned by WHO (viz. Quinolphos (0.01), DDE (1.00), Aldrin (0.05), Dieldrin (0.05), DDT (1.00), DDD (1.00), HCH (0.30) would be analysed for individual limits.

APPENDIX-I

1.1. APPARATUS FOR TESTS AND ASSAYS

1.1.1 Nessler Cylinders

Nessler cylinders which are used for comparative tests are matched tubes of clear colourless glass with a uniform internal diameter and flat, transparent base. They comply with Indian Standard 4161-1967. They are of transparent glass with a nominal capacity of 50 ml. The overall height is about 150 mm, the external height to the 50 ml mark 110 to 124 mm, the thickness of the wall 1.0 to 1.5 mm and the thickness of the base 1.5 to 3.0 mm. The external height to the 50 ml mark of the cylinder used for a test must not vary by more than 1 mm.

1.1.2 Sieves

Sieves for pharmacopoeial testing are constructed from wire cloth with square meshes, woven from wire of brass, bronze, stainless steel or any other suitable material. The wires should be of uniform circular cross-section and should not be coated or plated. There must be no reaction between the material of the sieve and the substance being sifted.

Sieves conform to the following specifications –

Approximate sieve number aperture size*	*Nominal mesh aperture size mm*	*Tolerance average ± mm*
4	4.0	0.13
6	2.8	0.09
8	2.0	0.07
10	1.7	0.06
12	1.4	0.05
16	1.0	0.03
—	µm	±µm
22	710	25
25	600	21
30	500	18
36	425	15
44	355	13
60	250	13(9.9)**
85	180	11(7.6)
100	150	9.4(6.6)

Approximate sieve number aperture size*	*Nominal mesh aperture size mm*	*Tolerance average ± mm*
120	125	8.1(5.8)
150	106	7.4(5.2)
170	90	6.6(4.6)
200	75	6.1(4.1)
240	63	5.3(3.7)
300	53	4.8(3.4)
350	45	4.8(3.1)

* Sieve number is the number of meshes in a length of 2.24 cm. In each transverse direction parallel to the wires.
** Figures in brackets refer to close tolerances, those without brackets relate to full tolerances.

1.1.3 Thermometers

Unless otherwise specified, thermometers suitable for pharmacopoeial tests conform to Indian Standard 4825-1968 and are standardised in accordance with the 'Indian Standard Method of Calibrating Liquid-in-Glass Thermometers', 6274-1971.

The thermometers are of the mercury-in-glass type and are filled with a dried inert gas, preferably nitrogen. They may be standardised for total immersion or for partial immersion. Each thermometer should be employed according to the condition of immersion under which it was standardised. In the selection of the theremometer it is essential to consider the conditions under which it is to be used.

1.1.4 Volumetric Glassware

Volumetric apparatus is normally calibrated at 27°. However, the temperature generally specified for measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25°. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27°.

Pharmacopoeial assays involving volumetric measurements require the use of accurately calibrated glassware. Volumetirc apparatus must be suitably designed to assure accuracy. The design, construction and capacity of volumetric glassware should be in accordance with those laid down by the Indian Standards Institution. The tolerances on capacity for volumetric flasks, pipettes and burettes, as laid down in the relevant Indian Standards, are set out in the following table.

Volumetric Flask : I.S. 915-1975								
Nominal capacity, ml	5	10	25	50	100	250	500	1000
Tolerance, ± ml	0.02	0.02	0.03	0.04	0.06	0.1	0.15	0.2

One Mark Pipettes : I.S. 1117 -1975								
Nominal capacity, ml	1	2	5	10	20	25	50	100
Tolerance, ± ml	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.06

Graduated Pipettes : I.S. 4162-1967					
Nominal capacity, ml	1	2	5	10	25
Subdivision, ml	0.01	0.02	0.05	0.10	0.2
Tolerance, ± ml	0.006	0.01	0.03	0.05	0.1

Burettes : I.S. 1997 – 1967				
Nominal capacity, ml	10	25	50	10
Subdivision, ml	0.05	0.05	0.1	0.1
Tolerance, ± ml	0.01	0.03	0.05	0.1

1.1.5 Weights and Balances

Pharmacopoeial tests and assays require the use of analytical balances that vary in capacity, sensitivity and reproducibility. The accuracy needed for a weighing should dictate the type of balance. Where substances are to be "accurately weighed", the weighing is to be performed so as to limit the error to not more than 0.1 per cent. For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and quantity of 10 g is to be weighed to the nearest 10 mg. A balance should be chosen such that the value of three times the standard deviation of the reproducibility of the balance, divided by the amount to be weighed, does not exceed 0.001.

APPENDIX-2

2.1. TESTING OF DRUGS

2.1.1. Systematic Study of Crude Drugs

In the Indian Systems of Medicine comprising of Ayurveda, Unani and Siddha, drugs of plant, animal and mineral origin, are used in their natural or so called "Crude" forms singly or in their mixture or in combination, to make a compound preparation of formulation. Nearly 90 per cent of the Crude Drugs are obtained from the plant sources while about 10 per cent of the drugs are derived from animal and mineral sources. The drugs of plant origin especially of herbaceous nature are frequently used as whole plant; otherwise their parts such as Root, Stem, Leaf, Flower, Seed, Fruit modifications of Stem and Root, Bark of a Stem or Root, Wood, and their Exudates or Gums etc. constitute single drugs in the Indian Systems of Medicine. These vegetable drugs are either used in dried forms or some times as whole fresh or their juice. The study of these crude drugs made with a view to recognise them is called Pharmacognosy (Pharmakon = Drug; Gignosco = to acquire knowledge of), meaning the knowledge or science of Drugs. In Pharmacognosy a complete and systematic study of a drug is done, which comprises of (I) origin, common names, scientific nomenclature and family, (ii) geographical source (and history), (iii) cultivation, collection, preservation and storage, (iv) Macroscopical, Microscopical and sensory (organoleptic) characters, (v) Chemical composition wherever possible, (vi) Identity, Purity, Strength and Assay, (vii) substitute and adulterants etc. Such systematic study of a drug as complete as possible is claimed to be the scientific or pharmacognositical evaluation.

As mentioned above each crude drug derived from the vegetable kingdom consists of a definite part of plant e.g., leaf, stem, fruit, seed, wood, bark, root etc. Morphological or Macroscopical details of the respective part are given by observing it with a naked eye or with the aid of a magnifying lens. In this description general conditions of the drug, size, shape, outer surface, inner surface etc are referred to. Drugs can be identified with the aid of the above, only if they are available in entire condition. Sensory or Organoleptic characters describe colour, odour, taste, consistency etc. The microscopic examination of different parts of the drug provides several diagnostic characters. In case of leaves, surface preparation and transverse section, preferably through midrib, are made and nature of epidermis, trichomes, stomata, arrangement of tissues like palisade cells, vascular bundles and nature of cell content are studied. Similarly in case of bark, root, rhizome and wood, transverse and longitudinal sections are made and from characteristic arrangements of tissues of each drug and from diagnostic elements like stone cells, fibres, vessels etc. as also from the study of the cell deposits like crystals, starch etc., the drugs are identified. The studies of diagnostic elements are helpful especially when the drugs are in powdered condition and give clues in the identification of drugs. Linear measurements and other methods of quantitative microscopy give further aid in the identification of the drugs. The sections or the powdered drugs samples are cleared by clearing agents, mostly chloral-hydrate solution, before mounting on the slide.

The basic chemical nature of cell-wall of almost all the plants is cellulosic, However, lignin, suberin, cutin or mucilage are deposited on the cellulose. Cellulose

gives blue colour with chlorzinc-iodine solution or with cuoxam. (Copper-oxide-ammonia) reagent. Lignin present in the middle lamella and secondary cell-well of many vessels, fibres and sclerieds gives red colour with phloroglucinol and concentrated hydrochloric acid. Suberin is present in cork and endodermis cells while cutin in the cuticle of leaf. Both are fatty in nature and when heated with Sudan Red-III give red colour.

Mucilage gives red colour with ruthenium red. The chemical constituents present in the drugs can be identified by chemical or microchemical tests e.g., Rhubarb rhizomes give with 5% potassium hydroxide red colour because of anthraquinone derivatives, strychnine present in Nux-vomica gives purplish-red colour with ammonium vanadate and concentrated sulphuric acid.

Paper and Thin Layer Chromotography are now utilised in identification of drugs, their adulterant and their chemical constituents. Methods have been developed for quantitative estimation of the chemical constituents from Paper and Thin Layer Chromatography (TLC).

2.1.2. Microscopical Methods of Examining Crude Vegetable Drugs

Methods of preparing specimens of crude materials of vegetable drugs for microscopical studies vary, depending on the morphological groups of drugs to be examined and also on the natures of the material i.e., entire, cut or powdered.

I. LEAVES, HERBS AND FLOWERS

For examining leaves, herbs and flowers (entire or cut) under microscope, following methods are employed for clarification:

A. Entire and cut materials

(i) *Entire materials* – When examining entire leaves, herbs and flowers, take pieces of leaf (margin and vein of leaves only), herbs (only leaf) and flowers (only calyx and corolla) in test tube. Add a solution of caustic alkali or nitric acid to the test tube and boil for 1-2 minutes, pour the contents into a porcelain dish, drain off the liquid, wash the material with water and leave for sometimes. Remove the pieces of the material from the water with a spatula and put on the slide, add a few drops of the solution of *glycerol or chloral hydrate.* Crush the material with scalpel and cover with cover slip before examining.

(ii) *Cut materials* – For examining cut leaves, herbs and flowers, take several pieces in a test tube and employ the same methods as described for entire materials.

Other methods employed for clarification of the material (leaf and stem) are described below :-

(a) **Leaf** – Boil pieces of leaves in a test tube with chloral hydrate for several minutes until completely clarified and then examine them in chloral hydrate solution. After clarification, leaf pieces are divided into two parts with the help of a scalpel or needle, and carefully turn one part. The leaf can be examined from both the dorsal and ventral surfaces.

(b) **Stem** – To examine stem material (without leaf) boil pieces in a solution of

caustic alkali or in *nitric acid.* Remove the epidermis with a scalpel or a needle for examining the surface. For examining pressed specimen of stem, take separate tissue and press them with a scalpel on the slide.

B. Powder

For examining characters of the powder take sufficient amount of powder in Chloral-hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time.

II. FRUITS AND SEEDS

A. Entire materials

For microscopical examination of fruit and seed take the specimens or outer coat of seed or fruit and examine as described below :

(i) *Outer Coat* – For examining the outer coat boil 3 or 4 seeds or fruits in caustic alkali solution in a test tube for 1-2 minutes (outer coat specimens with intensive pigmentation are boiled for longer period). After boiling, place the pieces on slide, remove the layers of the coat and examine them after mounting in glycerol solution.

(ii) *Section* – If fruits or seeds are too hard to cut then boil them for 15-30 minutes or more depending on their hardness or keep them in moistening chamber or absorb in water and chloroform solution or soften them with stem and then cut the specimen for examining purpose. For cutting small, flat seeds (which are difficult to hold) place them in a pith or potato slit for section cutting. Small, round or smooth seeds cannot be cut into section in the pith, then in such cases, they may be embedded in paraffin wax blocks for section cutting. For this, a block of paraffin (0.6 × 0.5 × 1.5 cms. in size) is made and the seed is embedded in the block by making a cavity or a pit in the block with a hot teasing needle. Cut the section with a sharp razor (through the object) together with the paraffin, place them on to the slide, remove paraffin with a needle or wash it with xylene and examine the section in *chloral-hydrate solution.*

B. Powder

For examining the structure of the cells of the seed coat and the cells of the embryo take a small amount of powder of the material on a slide in glycerol and cover it with a cover slip and examine.

1. *Starch* – For examining the presence of starch in the seed, take two specimens, one in iodine solution and the other in water. With iodine solution starch turns blue. Shape and the structure of starch grains can be seen in water and their size is measured.

When examining objects containing starch, prepare specimen by slightly warming in chloral-hydrate solution.

2. *Fixed Oil* – For examining the presence of fixed oil, prepare a specimen in a solution of Sudan III droplets of fixed oil are coloured orange pink. When examining

objects containing small amount of fixed oil, prepare a specimen by slightly warming in chloral-hydrate solution, and when examining objects containing large amount of fixed oil, then the powder is de-fatted and clarified as follows :

Place 0.5 g. of the powder in a porcelain dish, add 5-10 ml. of dilute nitric acid and boil for 1 minute, then strain off the liquid through a cloth, wash the residue with hot water and return it to the porcelain dish with a spatula, boil it with 5-10 ml of *caustic alkali solution* for 1 minute and again strain it through the cloth and wash with water. Examine the residue in a glycerol solution, after the treatment the structure of the layers of the coat and their cells can be seen very distinctly.

3. *Mucilage* – Prepare a specimen in Ruthenium Red and examine it under a low power microscope or under dissecting microscope. Mucilage appears as pinkish-red or yellow coloured masses.

III. BARKS

A. Entire material

Prepare transverse or longitudinal section of bark. To soften bark break it into pieces of about 1-2 cm long and 0.5-1 cm wide and boil with in a test tube for 1-3 minutes. Soft pieces are then straightened with a scalpel so as to have a exact transverse or longitudinal direction. Cut the section with razor, moisten the surface of the bark with glycerol solution. Remove the sections with a brush and place them on the slide. Thin pieces of the bark are cut by placing them in the pith (potato or carrot). The sections are treated with various reagents before examining.

1. *Lignified elements* – For testing lignin add several drops of *phloroglucinol* and a drop of *concentrated hydrochloric acid* to the section on a slide then draw off the liquid, immerse the section in *chloral hydrate solution* and cover with a cover slip (the specimen should not be heated); the lignified elements are coloured crimson. *Phloroglucinol* can be substituted by *saffranine*, and the lignified elements are coloured pink. The excessive stain can be washed out with acidified alcohol.

2. *Starch* – Starch is detected by treating with iodine solution.

3. *Tannin* –Tannin is detected by treating with *ferric ammonium sulphate solution* (blue-black or green black colour shows the presence of Tannin) or with *potassium-bi-chromate solution* (brown colour indicates the presence of Tannin).

4. *Anthraquinone derivatives* –Anthraquinone derivatives are detected by treating with alkali solution (blood-red colour shows the presence of anthraquinone derivatives).

B. Cut materials

Prepare small pieces or scraping of bark and boil them for 3-5 minutes in a solution of *caustic alkali* or *potassium hydroxide* or in *nitric acid solution* and then mount in *glycerin* for examination on a slide covered with a cover slip.

C. Powder

Prepare specimen for examination by placing a little amount of powder on a slide, add 1-2 drops of *phloroglucinol* and a drop of concentrated *hydrochloric acid*, cover

it with a cover slip, draw off the liquid from one side of the slide with filter paper, and then apply 1-2 drops of *chloral-hydrate solution* from the other side of the slide, lignified elements are stained crimson-red. Specimen may also be prepared with *caustic alkali or ferric ammonium sulphate* for this purpose.

IV. ROOTS AND RHIZOMES

A. Entire materials

For anatomical examination of entire roots and rhizomes cut transverse and longitudinal sections. For this, soften small pieces of roots without heating in *glycerol solution* for 1-3 days, depending on their hardness. The softened roots are straightened with the help of a scalpel in the right direction and then cut a section with the razor. First, cut thicker entire slices and then make thin, smaller sections. Stain the entire slices with *phloroglucinol* and *concentrated hydrochloric acid* or with *safranin* examine the specimen under a dissecting microscope. For micro-chemical test the small and thin sections are examined under microscope, as follows :

1. *Starch* – Starch is detected with iodine solution. For this, prepare specimen with water to measure the granule of starch with an occular micrometer.

2. *Inulin* –Inulin is detected with Molish's reagent. For this place a little powder on a slide and apply 1-2 drops of naphthol and a drop of concentrated sulphuric acid, if inulin is present, the powder will appear reddish-violet coloured. Starch also gives this test, so the test for inulin can be done in the absence of starch.

3. *Lignified elements* –Lignified elements (fibrovascular bundles, mechanical tissue etc.) are detected with *phloroglucinol* and concentrated *hydrochloric acid* or *safranine solution* as mentioned above for barks.

4. *Fixed oil* –For fixed oil detection use Sudan IV, as mentioned above for fruits and seeds.

If required for tannin, anthraquinone derivatives test as mentioned above.

B. Cut material

Make small pieces or scrapping of roots or rhizomes and boil them for 3-5 minutes in *caustic alkali,* or in *nitric acid* and then make pressed specimen and immerse them in *glycerol.*

Microchemical tests can be performed with scrapings for various chemicals as mentioned above.

C. Powder

Prepare several specimens of the powder on slides in *chloral hydrate solution* and perform the above mentioned standard tests for detection of starch, fixed oil, inulin, lignified elements, anthraquinone derivatives, tannins, mucilage, etc.

2.1.3. Types of Stomata

There are several types of stomata, distinguished by the form and arrangement

of the surrounding cells. The following descriptions apply to mature stomata.

1. Anomocytic (irregular-celled) –Previously known as ranunculaceous. The stoma is surrounded by a varying number of cells in no way differing form those of the epidermis generally.

2. Anisocytic (unequal-celled) –Previously known as cruciferous or solanaceous. The stoma is usually surrounded by three subsidiary cells, of which one is markedly smaller than the others.

3. Diacytic (cross-celled) –previously known as caryophyllaceous. The stoma is accompanied by two subsidiary cells whose common wall is at right angles to the guard cells.

4. Paracytic (parallel-celled) –Previously known as rubiaceous. The stoma has one each side one or more subsidiary cells parallel to the long axis of the pore and guard cells.

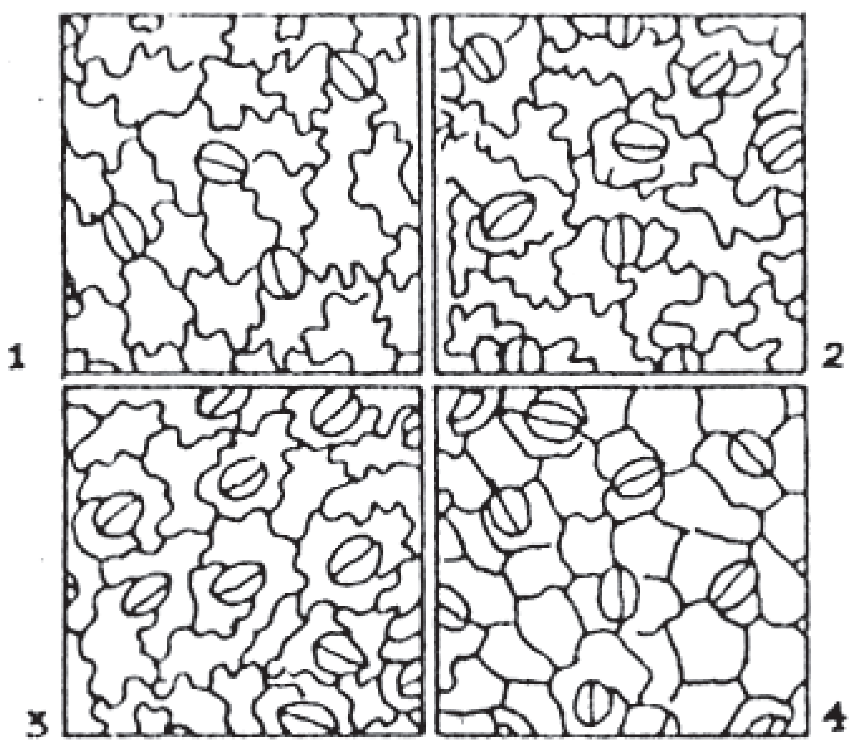


Fig. 1 Various types of stomata

2.1.4. Determination of Stomatal Index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

Place leaf fragments of about 5 × 5 mm in size in a test tube containing about 5 ml of *chloral hydrate solution* and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopic slide and prepare the mount, the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover-glass to prevent

the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each epidermal cell and a circle (o) for each stoma. Calculate the result as follows:

$$\text{Stomatal index} = \frac{S \times 100}{E + S}$$

Where S = the number of stomata in a given area of leaf ; and E = the number of epidermal cells (including trichomes) in the same area of leaf.

For each sample of leaf make not fewer than ten determinations and calculate the average index.

2.1.5. Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells under one epidermal cell.

Place leaf fragments of about 5 × 5 mm in size in a test-tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopical slide and prepare the mount of the upper epidermis in chloral hydrate solution and put a small drop of glycerol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Trace four adjacent epidermal cells on paper; focus gently downward to bring the palisade into view and trace sufficient palisade cells to cover the area of the outlines of the four epidermal cells. Count the palisade cells under the four epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4; this is the "Palisade ratio" (See Fig. 2).

For each sample of leaf make not fewer than ten determinations and calculate the average number.

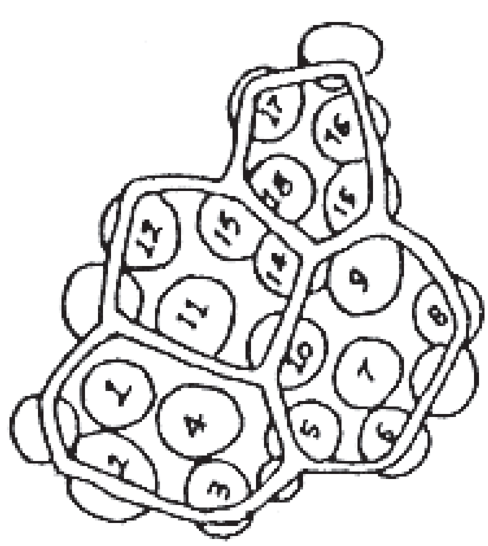


Fig. 2 Palisade ratio $\frac{18.4}{4} = 4.5$

2.1.6. Determination of Vein-Islet Number

The mesophyll of a leaf is divided into small portions of photosynthetic tissue by anastomosis of the veins and veinlets; such small portions or areas are termed "Vein-Islets". The number of vein-islets per square millimeter is termed the "Vein-Islet number". This value has been shown to be constant for any given species and, for full-grown leaves, to be unaffected by the age of the plant or the size of the leaves. The vein-islet number has proved useful for the critical distinction of certain nearly related species. The determination is carried out as follows :

For Whole or Cut leaves –-Take pieces of leaf lamina with an area of not less than 4 square millimeters from the central portion of the lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing c*hloral hydrate solution* on a boiling water-bath for 30 to 60 minutes or until clear and prepare a mount in *glycerol-solution* or, if desired, stain with *safranin solution* and prepare the mount in *Canada Balsam.* Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the centre of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make not fewer than three determinations and calculate the average number of vein-islets per square millimeter.

For Leaf Fragments having an area less than 4 square millimeters – Take fragments of leaf lamina each with an area of not less than 1 square millimeter, excluding the midrib and the margin of the leaf. Clear and prepare a mount as stated above. Use a 10x objective and a 6x eyepiece and draw a line representing 1 mm on a sheet of paper by means of a microscopial drawing apparatus and construct a square on this line representing an area of 1 square millimetre. Carry out the rest of the procedure as stated above. The result obtained is the number of vein-islets in 1 square millimetre. For each sample of leaf make no less than 12 determinations and calculate the average number.

2.1.7. Determination of Stomatal Number

Place leaf fragments of about 5x5 mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragments to a microscopic slide and prepare the mount the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with a 40 x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each stomata and calculate the average number of stomata per square millimetre for each surface of the leaf.

2.2. DETERMINATION OF QUANTITATIVE DATA OF VEGETABLE DRUGS

2.2.1. Sampling of Vegetable Drugs

Original Samples

(a) Samples of crude vegetable drugs in which the component parts are 1 cm or less in any dimension; and of powdered or ground drugs may be taken by means of sampling device that removes a core from the top to the bottom of the container. Not less than two cores are taken in opposite directions.

When the total weight of the drug to be sampled is less than 100 Kg, at least 250 g are withdrawn to constitute an original sample.

When the total weight of the drug to be sampled is more than 100 Kg, several samples are taken in the manner described, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until each of the quarters weigh at least 125 g; two such quarters then constitute an original sample.

(b) Samples of crude vegetable drugs in which the component parts are over 1 cm in any dimension may be taken by hand.

When the total weight of the drug to be sampled is less than 100 Kg, samples are taken from different parts of the container or containers. Not less than 500 g of samples so taken constitute an original sample.

When the total weight of the drug to be sampled is more than 100 Kg, several samples are taken in the manner described, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until each of the quarters weigh not less than 250 g; two such quarters then constitute an original sample.

Note:- Where the total weight of crude drug to be sampled is less than 10 Kg, the preceding methods may be followed but somewhat smaller quantities are to be withdrawn but in no case shall the original samples weight less than 125 g.

Test sample

Withdraw as much as may be necessary of the original sample by quartering, taking care to see that the portion is representative of the gross sample. In the case of unground or unpowdered drugs, grind the sample so that it will pass through a No. 22 sieve. If the sample cannot be ground, it should be reduced to as fine a state as possible. Mix by rolling it in paper or cloth, spread it out in a thin layer, and withdraw the portion for analysis.

2.2.2. Foreign Matter and Determination of Foreign Matter

A. FOREIGN MATTER

Drugs should be free from moulds, insects, animal faecal matter and other

contaminations such as earth, stones and extraneous material.

Foreign matter is material consisting of any or all of the following :-

(1) In particular, parts of the organ or organs from which the drug is derived other than the parts named in the definition or for which a limit is prescribed in the individual monograph.

(2) Any organ or part of organ, other than those named in the definition and description.

The amount of foreign matter shall not be more than the percentage prescribed in the monograph.

B. DETERMINATION OF FOREIGN MATTER

Weigh 100 –500 g of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x). Separate and weigh it and calculate the percentage present.

2.2.3. Determination of Total Ash

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°. Calculate the percentage of ash with reference to the air-dried drug.

2.2.4. Determination of Acid Insoluble Ash

Boil the ash obtained in (2.2.3) for 5 minutes with 25 ml of *dilute hydrochloric acid;* collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

2.2.5. (A) Determination of Water Soluble Ash

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temprature not exceeding 450°. Substract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

2.2.5. (B) Determination of Sulphated Ash

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 1 to 2 g of the substance, accurately weighed, into the

crucible, ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid,* heat gently until white fumes are no longer evolved and ignite at 800° ± 25° until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of *sulphuric acid* and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighings do not differ by more than 0.5 mg.

2.2.6. Determination of Alcohol Soluble Extractive

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.2.7. Determination of Water Soluble Extractive

Proceed as directed for the determination of Alcohol-soluble extractive, using *chloroform water* instead of ethanol.

2.2.8. Determination of Ether Soluble Extractive (Fixed Oil Content)

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with *Solvent ether* (or petroleum *ether,* b.p. 40° to 60°) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105° to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

2.2.9. Determination of Moisture Content (Loss on Drying)

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or unpowderdd drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.

Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

2.2.10. Determination of Volatile Oil in Drugs

The determination of volatile oil in a drug is made by distilling the drug with a mixture of *water* and *glycerin,* collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w.

The apparatus consists of the following parts (See Fig. 3). The apparatus described below is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.

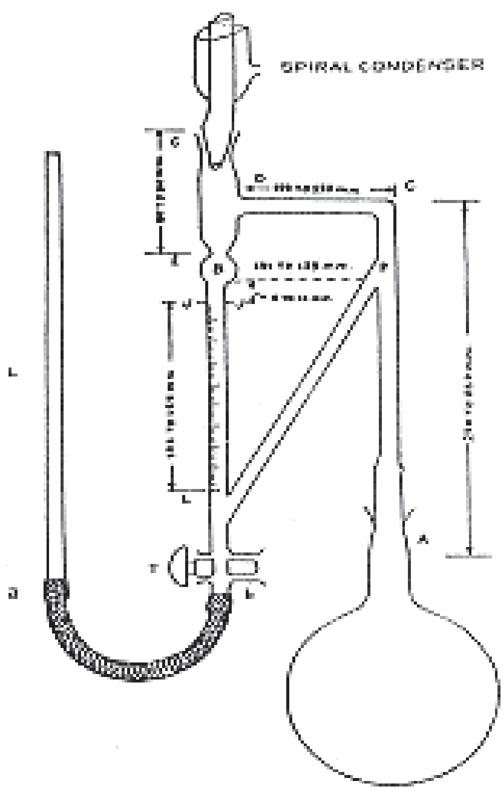


Fig. 3 Apparatus for volatile oil determination

Distilling Flask – A spherical flask, 1,000 ml capacity with ground neck, taper of ground socket 1 in 10, internal diameter of larger end 34.35 to 34.65 mm

(a) **Still head** –graduated measuring tube, and return flow tube made in one piece, in accordance with the following specifications. External diameter of the smaller end 31.0 to 31.2 mm. Minimum length of the ground zone –34 mm.

Tube AC, length –220 to 240 mm.
Internal diameter –13 to 15 mm.

Bulb CD, length –100 to 110 mm.
Internal diameter –13 to 15 mm.

Spiral condenser –ground joint accurately fitting in the ground neck of the tube EG, taper 1 in 10.

Tube EG, length –80 to 90 mm.
Internal Diameter –30 to 40 mm.

Bulb B –length 20 to 22 mm.
Internal diameter –15 to 20 mm.

The distance between B and P is 120 to 125 mm.

Junction P and the centre of the bulb B must be in the same horizontal plane.

Measuring tube JL – length of the graduated portion 144 to 155 mm capacity 2 millilitres graduated into fifths and fiftieths of a millilitre.

Tube PL – return flow tube –Internal diameter –7 to 8 mm. Levelling tube I, length –450 to 500 mm. Internal diameter 10 to 12 mm tapering at the lower end with a wide top (20 to 25 mm diameter).

Rubber tubing a—b length 450 to 500 mm. Internal diameter 5 to 8 mm.

(b) **Burner** –A luminous Argand burner with chimney and sensitive regulative tap.

(c) **Stand** –A retort stand with asbestos covered ring and clamp carrying a piece of metal tubing connected by a short length of rubber tubing with the water inlet tube of the condenser jacket.

The Whole of the apparatus is effectively screened from draught.

The apparatus is cleaned before each distillation by washing successively with *acetone* and *water,* then inverting it, filling it with *chromic sulphuric acid* mixture, after closing the open end at G, and allowing to stand, and finally rinsing with water.

Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml of *glycerin* and 175 ml of *water* in the one litre distilling flask, and a few pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into the graduated receiver, keeping the tap T open until the water overflows, at P. Any air bubbles in the rubber tubing a—b are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate, which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap T is opened and the tube L_1 lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read.

The tube L_1 is then raised till the level of water in it is above the level of B, when the tap T is slowly opened to return the oil to the bulb. The distillation is again

continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug. The dimensions of the apparatus may be suitably modified in case of necessity.

2.2.11. SPECIAL PROCESSES USED IN ALKALOIDAL ASSAYS

2.2.11.a Continuous extraction of drug –

Where continuous extraction of a drug of any other substance is recommended in the monograph, the process consists of percolating it with a suitable solvents at a temperature approximately that of the boiling point of the solvent. Any apparatus that permits the uniform percolation of the drug and the continuous flow of the vapour of the solvent around the percolator may be used. The type commonly known as the Soxhlet apparatus is suitable for this purpose.

A simple apparatus is shown in the accompanying illustraion. A is an outer tube of stout glass; the wider part is about 18 cm in length and has an internal diameter of 4.8 to 5 cm; the lower end C is about 5 cm in length and has an external diameter of about 1.6 cm. B is a straight glass tube open at both ends, about 9 cm in length and having an external diameter of about 3.8 cm; over its lower flanged end is tied firmly with a piece of calico or other suitable material. D is a glass coil, which supports the margin of the tube B and prevents it from resting in contact with the outer tube A. The lower end C of the outer tube A is fitted by a cork to the distilling flask E, in which a suitable quantity of the solvent has been placed. The substance to be extracted, previously moistened with the solvent or subjected to any preliminary treatment required, is introduced into the inner tube B, which is supported so that the percolate drops into the outer tube. A pad of cotton wool G is placed on the top of the drug, the inner tube is lowered into position and the outer tube connected by means of a suitable cork with the tube of a reflux condenser F. The flask is heated and the extraction continued as directed (See Fig. 4).

2.2.11.b TESTS FOR COMPLETE EXTRACTION OF ALKALOIDS –

Complete extraction is indicated by the following tests :

When extracting with an aqueous or alcoholic liquid –After extracting at least three times with the liquid, add to a few drops of the next portion, after acidifying with 2 *N hydrochloric acid* if necessary, 0.05 ml of potassium mercuri-iodide solution or for solanaceous alkaloids 0.05 ml of *potassium iodobismuthate solution;* no precipitate or turbidity, is produced.

When extracting with an immiscible solvent –After extracting at least three times with the solvent, add to 1 to 2 ml of the next portion 1 to 2 ml of 0.1 *N hydrochloric acid,* remove the organic solvent by evaporation, transfer the aqueous residue to a test tube, and add 0.05 ml of *potassium mercuri-iodide solution* for solanaceous alkaloids 0.05 ml of *potassium iodobismuthate solution* or for emetine, 0.05 ml of *iodine solution;* not more than a very faint opalescenece is produced.

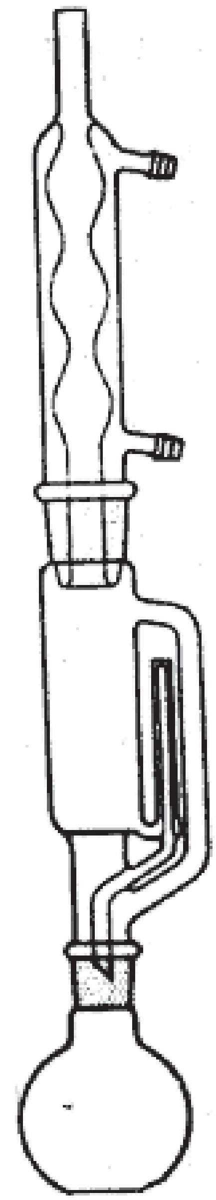


Fig. 4 Apparatus for the continuous extraction of Drugs

2.2.12. Thin-Layer Chromatography (TLC)

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Apparatus

(a) Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively, commercially prepared plates may be used.

(b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

(c) The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 µm to 40 µm in diameter is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of Plaster of Paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.

(d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

(e) A storage rack to support the plates during drying and transportation.

(f) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.

(g) Graduated micro-pipettes capable of delivering microlitre quantities say 10 µl and less.

(h) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

(i) An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of plates –Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100° to 105° for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs.

Method

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow

strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specified 'protected from light' or 'in subdued light' it is intended that the entire procedure is carried out under these conditions.

Visualisation

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (365 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term *secondary spot* means any spot other than the principal spot. Similarly, a *secondary band* is any band other than the principal band.

Rf. Value

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

2.2.13. Starch estimation (Mont Gomery 1957) [Spectrophotometric method]

Prepare 10% homogenate of the plant tissue in 80% Ethanol. Centrifuge at 2000 rpm for 15 minutes. To the residue thus obtained, add 4 ml of distilled water, heat on a water bath for 15 minutes and macerate with the help of glass rod. To each of the samples, add 3 ml of 52% perchloric acid and centrifuge at 2000 rpm for 15 minutes. The supernatant thus obtained is made upto known volume (generally upto 10 ml or depending on the expected concentration of starch). Take 0.1 ml aliquot, add 0.1 ml of 80% phenol and 5 ml conc. H_2SO_4. Cool and then read the absorbance at 490 nm.

2.2.14. Sugar estimation (Mont Gomery 1957) [Spectrophotometric method]

Prepare 10% homogenate of the plant tissue in 80% Ethanol. Centrifuge at 2000 rpm for 15 minutes. The supernatant obtained is made upto known volume (generally

upto 10 ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot, add 0.1 ml of 80% phenol and 5 ml conc. H_2SO_4. Cool and then read the absorbance at 490 nm.

2.2.15. Fatty oil estimation

To estimate fatty oils, extract accurately weighed air dried powdered plant material with petroleum ether (40-60^{0}C) in Soxhlet apparatus. Dry the extract over anhydrous sodium sulphate and remove the solvent under vacuum at 40^{0}C. Weigh the residue and calculate the percentage with reference to the weight of plant material used.

2.2.16. Determination of foaming index

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 150), weigh accurately and transfer to a 500-ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100-ml volumetric flask and add sufficient water through the filter to dilute the volume to 100 ml.

Place the above decoction into 10 stoppered test-tube (height 16 cm. diameter 16 mm) in a series of successive portions of 1, 2, 3, upto 10 ml and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, 2 frequencies per second. Allow to stand for 15 minutes. Note 1 cm height of the foam and calculate the foaming index by following formula.

$$\text{Foaming index} = \frac{1000}{a}$$

Where is the volume in ml, of decoction used for preparing dilution in tube where foaming is observed.

2.2.17. Protein estimation (Lowry et al 1951)

Homogenise 100 mg plant material with 3 ml of 10% Trichloroacetic acid. Centrifuge the homogenate at 10,000 rpm. discard the supernatant. Treat the pellets obtained after centrifugation with 3 ml 1N NaOH, heat on water bath for 7 minutes and cool. Centrifuge the solution again for five to ten minutes at 5000 rpm. To 0.5 ml of supernatant thus obtained after centrifugation, add 5 ml reagent containing 100 parts of 2% solution of sodium carbonate and one part of 2% solution of sodium potassium tartrate. Allow it to stand for ten to fifteen minutes. Then add 5 ml Folin and Ciocalteu's Phenol reagent (diluted with distilled water in ratio of 1:1) and allow to stand for half-hour for development of colour and then finally measure the absorbance at 700 nm.

2.2.17. (A) Isolation of Forskohlin (Shah *et al,* 1980)

Extract the powdered air dried roots (500 g) in percolator at room temperature successively with petroleum ether (60 – 80^0) [3x2L] and ethyl alcohol [4 x 2L]. Concentrate the petroleum ether and the ethyl alcohol extracts under reduced pressure to give 10 g of petroleum ether extractive and 22 g of ethyl alcohol extractive. Combine the two

extractives and fraction it first with hexane [4 x 250 ml] and then with benzene [5 x 250 ml]. A dark brown material will be obtained. Dry it under vacuum and subject to column chromatography over silica gel [300 g] using benzene with increasing amount of ethyl acetate, in order of 20%, as eluent. Collect fractions, of 100 ml and check for coleonol by TLC (Benzene : Methanol :: 95 : 5). Fractions 1–40 did not show spots corresponding to coleonol whereas fractions 41–42 were found to contain coleonol (0.15%).

2.2.18. Method for Alkaloid estimation

Macerate the plant material with 2% acetic acid in water, filter and concentrate the filtrate under reduced pressure at 45^{0}C to one third of the original volume. Adjust the pH to 2 by 4 M HCl. The yellow precipitate will be separated from the solution (A). Dissolve in it 0.1 M HCl to give solution (B). Add Mayer's reagent to the solution A and B to give precipitate of Alkaloid Mayer complex. Dissolve it again in acetone - methanol - water (6 : 2 : 10). to give solution of Alkaloid Mayer Complex. Pass this complex finally through Amberlite IRA 400 anion exchange resin (500 g) to give an aqueous solution of alkaloid chlorides.

2.3. LIMIT TESTS

2.3.1. Limit Test for Arsenic

In the limit test for arsenic, the amount of arsenic is expressed as arsenic, As

Apparatus

A wide-mouthed bottle capable of holding about 120 ml is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). It is drawn out at one end to a diameter of about 1 mm and a hole not less than 2 mm in diameter is blown in the side of the tube, near the constricted part. When the bung is inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid, and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly rounded or ground smooth.

Two rubber bungs (about 25 mm X 25 mm), each with a hole bored centrally and true, exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively the two bungs may be replaced by any suitable contrivance satisfying the conditions described under *the General Test.*

Reagents

Ammonium oxalate AsT : *Ammonium oxalate* which complies with the following additional test :

Heat 5 g with 15 ml of *water,* 5 ml of *nitric acid AsT,* and 10 ml of *Sulphuric acid AsT* in narrow necked, round-bottomed flask until frothing ceases, cool, and apply the General Test; no visible stain is produced.

Arsenic solution, dilute, AsT :

Strong Arsenic solution AsT	1 ml
Water sufficient to produce	100 ml
Dilute arsenic solution AsT must be freshly prepared.	
1 ml contains 0.01 mg of arsenic, As.	

Arsenic solution, strong, AsT :

Arsenic trioxide	0.132 g
Hydrochloric acid	50 ml
Water sufficient to produce	100 ml

Brominated hydrochloric acid AsT :

Bromine solution AsT	1 ml
Hydrochloric acid AsT	100 ml

Bromine solution AsT :

Bromine	30 g
Potassium bromide	30 g
Water sufficient to produce	100 ml

It complies with the following test :

Evaporate 10 ml on a water-bath nearly to dryness, add 50 ml of water, 10 ml of *hydrochloric acid AsT* and sufficient *stannous chloride solution AsT* to reduce the remaining bromine and apply the General Test; the stain produced is not deeper than 1 ml *standard stain,* showing that the proportion of arsenic present does not exceed 1 part per million.

Citric acid AsT : *Citric acid* which complies with the following additional tests : Dissolve 10 g in 50 ml of water add 10 ml of *stannated hydrochloric acid AsT* and apply the General Test; no visible stain is produced.

Hydrochloric acid AsT : *Hydrochloric acid* diluted with *water* to contain about 32 per cent w/w of HCI and complying with the following additional tests :

(i) Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of *ammonium thiocyanate solution* and stir immediately; no colour is produced.

(ii) To 50 ml add 0.2 ml of *bromine solution AsT,* evaporate on a water-bath until reduced to 16 ml adding more *bromine solution AsT,* if necessary, in order that an excess, as indicated by the colour, may be present throughout the evaporation; add 50 ml of *water* and 5 drops of *stannons chloride solution AsT,* and apply the General Test; the stain producted is not deeper than a 0.2 ml *standard stain* prepared with the same acid, showing that the proportion of arsenic present does not exceed 0.05 part per million.

Hydrochloric acid (constant-boiling composition) AsT : Boil *hydrochloric acid AsT to constant boiling Composition* in the presence of *hydrazine hydrate*, using 1 ml of 10 per cent w/v solution in *water* per litre of the acid.

Mercuric chloride paper – Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of *mercuric chloride,* pressed to remove superfluous solution, and dried at about 60º, in the dark. The grade of the filter paper is such that the weight is between 65 and 120 g per sq. mm; the thickness in mm of 400 papers is approximately equal numerically, to the weight in g per sq. mm.

Nitric acid AsT : *Nitric acid* which complies with the following additional test :

Heat 20 ml in a porcelain dish with 2 ml of *sulphuric acid AsT,* until white fumes

are given off. Cool, add 2 ml of water, and again heat until white fumes are given off; cool, add 50 ml of water and 10 ml of *stannated hydrochloric acid AsT,* and apply the General Test; no visible stain is produced.

Potassium chlorate AsT : *Potassium chlorate* which complies with the following additional test :

Mix 5 g in the cold with 20 ml of *water* and 22 ml of *hydrochloric acid AsT;* when the first reaction has subsided, heat gently to expel chlorine, remove the last traces with a few drops of *stannous chloride solution AsT,* add 20 ml of water, and apply the General Test; no visible stain is produced.

Note – murcuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia affords a lighter stain or no stain at all when empolyed in the limit test for arsenic.

Potassium iodide AsT : *Potassium iodide* which complies with the following additional test :

Dissolve 10 g in 25 ml of *hydrochloric acid AsT* and 35 ml of *water,* add 2 drops of *stannous chloride solution AsT* and apply the General Test; no visible stain is produced.

Sodium carbonate, anhydrous AsT : *Anhydrous sodium carbonate* which complies with the following additional test :

Dissolve 5 g in 50 ml of *water,* add 20 ml of *brominated hydrochloric acid AsT,* remove the excess of bromine with a few drops of *stannous chloride solution AsT,* and apply the General Test; no visible stain is produced.

Stannated hydrochloric acid AsT :

Stannous chloride solution AsT	1 ml
Hydrochloric Acid AsT	100 ml

Stannous chloride solution AsT : Prepared from *stannous chloride solution* by adding an equal volume of *hydrochloric acid*, boiling down to the original volume, and filtering through a fine-grain filter paper.

It complies with the following test :

To 10 ml add 6 ml of water and 10 ml of *hydrochloric acid AsT,* distil and collect 16 ml. To the distillate and 50 ml of *water* and 2 drops of *stannuous chloride solution AsT* and apply the General Test; the stain produced is not deeper than a 1-ml *standard stain,* showing that the proportion of arsenic present does not exceed 1 part per million.

Sulphuric acid AsT : *Sulphuric acid* which complies with the following additional test :

Dilute 10 g with 50 ml of water, add 0.2 ml of *stannous chloride solution AsT,* and apply the General Test; no visible stain is produced.

Zinc AsT : *Granulated zinc* which complies with following additional test :

Add 10 ml of *stannated hydrochloric acid AsT* to 50 ml of *water,* and apply the General Test, using 10 of the zinc and allowing the action to continue for one hour; no visible stain is produced (limit of arsenic). Repeat the test with the addition of 0.1 ml of *dilute arsenic solution AsT;* a faint but distinct yellow stain is produced (test for sensitivity).

General Method of Testing –- By a variable method of procedure suitable to the particular needs of each substance, a solution is prepared from the substance being examined which may or may not contain that substance, but contains the whole of the arsenic (if any) originally present in that substance. This solution, referred to as the 'test solution', is used in the actual test.

General Test – The glass tube is lightly packed with cotton wool, previously moistened with *lead acetate solution* and dried, so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube. The upper end of the tube is then inserted into the narrow end of one of the pair of rubber bungs, either to a depth of about 10 mm when the tube has a rounded-off end, or so that the ground end of the tube is flush with the larger end of the bung. A piece of *mercuric chloride paper* is placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band or spring clip in such a manner that the borings of the two bungs (or the upper bung and the glass tube) meet to form a true tube 6.5 mm in diameter interrupted by a diaphragm of *mercuric chloride paper.*

Instead of this method of attaching the *mercuric chloride paper,* any other method may be used provided (1) that the whole of the evolved gas passes through the paper; (2) that the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and (3) that the paper is protected from sunlight during the test. The test solution prepared as specified, is placed in the wide-mouthed bottle, 1 g of *potassium iodide AsT* and 10 g of *zinc AsT* added, and the prepared glass tube is placed quickly in position. The action is allowed to proceed for 40 minutes. The yellow stain which is produced on the *mercuric chloride paper* if arsenic is present is compared by day light with the *standard stains* produced by operating in a similar manner with known quantities of *dilute arsenic solution AsT .* The comparison of the stains is made immediately at the completion of the test. The *standard stains* used for comparison are freshly prepared; they fade on keeping.

By matching the depth of colour with *standard stains*, the proportion of arsenic in the substance may be determined. A stain equivalent to the 1-ml *standard stain*, produced by operating on 10 g of substance indicates that the proportion of arsenic is 1 part per million.

Note – (1) The action may be accelerated by placing the apparatus on a warm surface, care being taken that the mercuric chloride paper remains dry throughout the test.

(2) The most suitable temperature for carrying out the test is generally about 40° but because the rate of the evoluion of the gas varies somewhat with different batches zinc AsT, the temperature may be adjusted to obtain a regular, but not violent, evolution of gas.

(3) The tube must be washed with *hydrochloric acid AsT*, rinsed with water and dried between successive tests.

Standard Stains – Solutions are prepared by adding to 50 ml of water, 10 ml of *stannated hydrochloric acid AsT* and quantities of *dilute arsenic solutions AsT* varying from 0.2 ml to 1 ml. The resulting solutions, when treated as described in the General Test, yield stains on the *mercuric chloride paper* referred to as the standard stains.

Preparation of the Test Solution

In the various methods of preparing the test solution given below, the quantities are so arranged unless otherwise stated, that when the stain produced from the solution to be examined is not deeper than the 1-ml *standard stain,* the proportion of arsenic present does not exceed the permitted limit.

Ammonium chloride – Dissolve 2.5 g in 50 ml of *water,* and 10 ml of *stannated hydrochloric acid AsT.*

Boric acid – Dissolve 10 g with 2 g of *citric acid AsT* in 50 ml water, and add 12 ml of *stannated hydrochloric acid AsT.*

Ferrous sulphate – Dissolve 5 g in 10 ml of *water and 15 ml of stannated hydrochloric* acid *AsT and* disitil 20 ml; to the distillate add a few drops of *bromine solution AsT.* Add 2 ml of *stannated hydrochloric acid AsT,* heat under a reflux condenser for one hour, cool, and add 10 ml of *water* and 10 ml of *hydrochloric acid AsT.*

Glycerin – Dissolve 5 g in 50 ml of water, and add 10 ml of *stannated hydrochloric acid AsT.*

Hydrochloric acid – Mix 10 g with 40 ml of water and 1 ml of *stannous chloride solution AsT.*

Magnesium sulphate – Dissolve 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT.*

Phosphoric acid – Dissolve 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*

Potassium iodide – Dissolve 5 g in 50 ml of *water* and add 2 ml of *stannated hydrochloric acid AsT.*

Sodium bicarbonate – Dissolve 5 g in 50 ml of *water* and add 15 ml of *brominated hydrochloric acid AsT,* and remove the excess of bromine with a few drops of *stannous chloride solution AsT.*

Sodium hydroxide – Dissolve 2.5 g in 50 ml of *water,* add 16 ml of *brominated hydrochloric acid AsT,* and remove the excess of *bromine* with a few drops of *stannous chloride solution AsT.*

2.3.2. Limit Test for Chlorides

Dissolve the specified quantity of the substance in *water* or prepare a solution as directed in the text and transfer to a *Nessler cylinder.* Add 10 ml of *dilute nitric acid,*

except when nitric acid is used in the preparation of the solution, dilute to 50 ml with water, and add 1 ml of *silver nitrate solution.* Stir immediately with a glass rod and allow to stand for 5 minutes. The opalescence produced is not greater than the *standard opalescence,* when viewed transversely.

Standard Opalescence

Place 1.0 ml of a 0.05845 percent w/v solution of *sodium chloride* and 10 ml of *dilute nitric acid* in a *Nessler cylinder*. Dilute to 50 ml with water and add 1 ml of *silver nitrate solution*. Stir immediately with a glass rod and allow to stand for five minutes.

2.3.3. Limit Test for Heavy Metals

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution.

Determine the amount of heavy metals by one of the following methods and as directed in the individual monographs. Method A is used for substances that yield clear colourless solutions under the specified test conditions. Method B is used for substances that do not yield clear, colourless solutions under the test conditions specified for method A, or for substances which, by virtue of their complex nature, interfere with the precipitation of metals by sulphide ion. Method C is used for substances that yield clear, colourless solutions with *sodium hydroxide solutions.*

Special Reagents –

Acetic acid Sp. – *Acetic acid* which complies with the following additional test : Make 25 ml alkaline with *dilute ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with *water* and add two drops of *sodium sulphide* solution; no darkening is produced.

Dilute acetic acid Sp. – *Dilute acetic acid, which* complies with the following additional test – Evaporate 20 ml in a porcelain dish, nearly to dryness on a water-bath. Add to the residue 2 ml of the acid and dilute with water to 25 ml, add 10 ml of *hydrogen sulphide solution.* Any dark colour produced is not more than that of a control solution consisting of 2 ml of the acid and 4.0 ml of *standard lead solution* diluted to 25 ml with *water.*

Ammonia solution Sp. – *Strong ammonia solution* which complies with the following additional test : Evaporate 10 ml to dryness on a water-bath; to the residue add 1 ml of *dilute hydrochloric acid Sp. a*nd evaporate to dryness. Dissolve the residue in 2 ml of dilute acetic acid Sp. Add sufficient water to produce 25 ml.

Add 10 ml of *hydrogen sulphide solution.* Any darkening produced is not greater than in a blank solution containing 2 ml of dilute acetic acid Sp. 1.0 ml of *standard lead solution* and sufficient *water* to produce 25 ml.

Dilute ammonia solution Sp. – *Dilute ammonia solution* which complies with the following additional test : To 20 ml add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with *water,* and add two drops of *sodium sulphide solution;* no darkening is produced.

Hydrochloric acid – *Hydrochloric acid* which complies with the following additional test : Evaporate off the acid in a beaker to dryness on a water-bath. Dissolve the residue in 2 ml of *dilute acid Sp.,* dilute to 17 ml with water and add 10 ml of *hydrogen sulphide solution;* any darkening produced is not greater than in a blank solution containing 2.0 ml of *standard lead solution,* 2 ml of *dilute acetic acid Sp.* and dilute to 40 ml with water.

Dilute hydrochloric acid Sp. – *Dilute hydrochloric acid,* which complies with the following additonal test: Treat 10 ml of the acid in the manner described under *Hydrochloric acid Sp.*

Lead nitrate stock solution – Dissolve 0.1598 g of *lead nitrate* in 100 ml of *water* to which has been added 1 ml of *nitric acid,* then dilute with *water* to 1000 ml. This solution must be prepared and stored in polyethylene or glass containers free from soluble lead salts.

Standard lead solution – On the day of use, dilute 10.0 ml of *lead nitrate stock solution* with *water* to 100.0 ml. Each ml of *standard lead solution* contains the equivalent of 10 µg of lead. A control comparison solution prepared with 2.0 ml of standard lead solution contains, when compared to a solution representing 1.0 g of the substance being tested, the equivalent of 20 parts per million of lead.

Nitric acid Sp. –*Nitric acid* which complies with the following additional test : Dilute 10 ml with 10 ml of *water,* make alkaline with *ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with water, and add two drops of *sodium sulphide solution;* no darkening is produced.

Potassium cyanide solution Sp. – See Appendix 2.3.5.

Sulphuric acid Sp. – Sulphuric acid which complies with following additional test : Add 5 g to 20 ml of *water* make alkaline with *ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with *water* and add two drops of *sodium sulphide solution;* no darkening is produced.

Method A

Standard solution – Into a 50 ml *Nessler cylinder,* pipette 2 ml of *standard lead solution* and dilute with *water* to 25 ml. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml, and mix.

Test solution – Into a 50 ml *Nessler cylinder,* place 25 ml of the solution prepared for the test as directed in the individual monogrpah, or using the stated volume of acid when specified in the individual monograph, dissolve and dilute with *water* to 25 ml the specified quantity of the substance being tested. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp.* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml and mix.

Procedure – To each of the cylinders containing the *standard solution* and t*est* solution respectively add 10 ml of freshly prepared *hydrogen sulphide solution,* mix, dilute with *water* to 50 ml, allow to stand for five minutes, and view downwards over a white surfac; the colour produced in the *test solution* is not darker than that produced in the *standard solution.*

Method B

Standard solution – Proceed as directed under Method A.

Test solution – Weigh in a suitable crucible the quantity of the substance specified in individual monograph, add sufficient *sulphuric acid Sp.* to wet the sample, and ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of *nitric acid Sp.* and five drops of *sulphuric acid Sp.* and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500º to 600º until the carbon is completely burnt off . Cool, add 4 ml of *hydrochloric acid Sp.,* cover, digest on a water bath for 15 minutes, uncover and slowly evaporate to dryness on a water-bath. Moisten the residue with one drop of *hydrochloric acid Sp.,* add 10 ml of hot water and digest for two minutes. Add *ammonia solution Sp.,* dropwise, until the solution is just alkaline to *litmus paper,* dilute with *water* to 25 ml and adjust with dilute acetic acid Sp. to a pH between 3.0 and 4.0. Filter if necessary, rinse the crucible and the filter with 10 ml of water, combine the filtrate and washings in a 50 ml *Nessler cylinder,* dilute with *water,* to about 35 ml, and mix. Procedure : Proceed as directed under Method A.

Method C

Standard solution – Into a 50 ml *Nessler cylinder,* pipette 2 ml of *standard lead solution,* add 5 ml of *dilute sodium hydroxide solution.,* dilute with *water* to 50 ml and mix.

Test solution – Into a 50 ml *Nessler cylinder,* place 25 ml of the solution prepared for the test as directed in the individual monograph; or, if not specified otherwise in the individual monograph, dissolve the specified quantity in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution.* Dilute 50 ml with water and mix.

Procedure –To each of the cylinders containing the *standard solution* and the *test solution,* respectively add 5 drops of *sodium sulphide solution,* mix, allow to stand for five minutes and view downwards over a white surface; the colour produced in the *test solution* is not darker than that produced in the *standard solution.*

2.3.4. Limit Test for Iron

Standard iron solution – Weigh accurately 0.1726 g of *ferric ammonium sulphate* and dissolve in 10 ml of 0.1 *N sulphuric acid* and sufficient *water* to produce 1000.0 ml. Each ml of this solution contains 0.02 mg of Fe.

Method

Dissolve the specified quantity of the substance being examined in 40 ml of *water,* or use 10 ml of the solution precribed in the monograph, and transfer to a

Nessler cylinder. Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid,* mix, make alkaline with *iron-free ammonia solution,* dilute to 50 ml with *water* and allow to stand for five minutes. Any colour produced is not more intense than the standard colour.

Standard colour – Dilute 2.0 ml of *standard iron solution* with 40 ml of *water* in a *Nessler cylinder.* Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid,* mix, make alkaline with *iron-free ammonia solution,* dilute to 50 ml with *water* and allow to stand for five minutes.

2.3.5. Limit Test for Lead

The following method is based on the extraction of lead by solutions of dithizone. All reagents used for the test should have as low a content of lead as practicable. All reagent solutions should be stored in containers of borosilicate glass. Glassware should be rinsed thoroughly with warm *dilute nitric acid,* followed by *water.*

Special Reagents

(1) Ammonia-cyanide solution Sp. – Dissolve 2 g of *potassium cyanide* in 15 ml of *strong ammonia solution* and dilute with *water* to 100 ml.

(2) Ammonium citrate solution Sp. – Dissolve 40 g of *citric acid* in 90 ml *water.* Add two drops of phenol *red solution* then add slowly *strong ammonia solution* until the solution acquires a reddish colour. Remove any lead present by extracting the solution with 20 ml quantities of *dithizone extraction solution* until the dithizone solution retains its orange-green colour.

(3) Dilute standard lead solution – Dilute 10.0 ml of *standard lead solution* with sufficient 1 per cent v/v solution of nitric acid to produce 100.0 ml. Each ml of this solution contains 1 µg of lead per ml.

(4) Dithizone extraction solution – Dissolve 30 mg of *diphenylthiocarbazone* in 1000 ml of *chloroform* and add 5 ml of *alcohol.* Store the solution in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1 per cent v/v solution of *nitric acid* and discard the acid.

(5) Hydroxylamine hydrochloride solution Sp. – Dissolve 20 g of *hydroxylamine hydrochloride* in sufficient *water* to produce about 65 ml. Transfer to separator, add five drops of *thymol blue solution*, add *strong ammonia solution* until the solution becomes yellow. Add 10 ml of a 4 per cent w/v solution of *sodium diethyldithiocarbamate* and allow to stand for five minutes. Extract with successive quantities, each of 10 ml, of *chloroform* until a 5 ml portion of the extract does not assume a yellow colour when shaken with dilute copper sulphate solution. Add *dilute hydrochloric acid* until the solution is pink and then dilute with sufficient water to produce 100 ml.

(6) Potassium cyanide solution Sp. – Dissolve 50 g of potassium cyanide in sufficient *water* to produce 100 ml. Remove the lead from this solution by extraction with successive quantities, each of 20 ml of *dithizone extraction solution* until the dithizone solution retains its orange-green colour. Extract any dithizone remaining in the

cyanide solution by shaking with *chloroform.* Dilute this cyanide solution with sufficient *water* to produce a solution containing 10 g of *potassium cyanide* in each 100 ml.

(7) **Standard dithizone solution** – Dissolve 10 ml of *diphenylthiocarbazone* in 1000 ml of *chloroform.* Store the solution in a glass-stoppered, lead-free bottle, protected from light and in a refrigerator.

(8) **Citrate-cyanide wash solution** – To 50 ml of *water* add 50 ml of *ammonium citrate solution Sp.* and 4 ml of *potassium cyanide solution Sp.,* mix, and adjust the pH, if necessary, with strong *ammonia solution* to 9.0.

(9) **Buffer solution pH 2.5** – To 25.0 ml of 0.2 *M potassium hydrogen phthalate add* 37.0 ml of 0.1 N *hydrochloric acid,* and dilute with sufficient *water* to produce 100.0 ml.

(10) **Dithizone-carbon tetrachloride solution** –Dissolve 10 mg of *diphenylthiocarbazone* in 1000 ml of carbon tetrachloride. Prepare this solution fresh for each determination.

(11) **pH 2.5 wash solution** – To 500 ml of a 1 per cent v/v *nitric acid* add *strong ammonia solution* until the pH of the mixture is 2.5, then add 10 ml of *buffer solution* pH 2.5 and mix.

(12) **Ammonia-cyanide wash solution** – To 35 ml of pH 2.5 *wash solution* add 4 ml of *ammonia-cyanide solution Sp.,* and mix.

Method

Transfer the volume of the prepared sample directed in the monograph to a separator and unless otherwise directed in monograph, add 6 ml of *ammonium citrate solution Sp.,* and 2 ml *hydroxylamine hydrochloride solution Sp.,* (For the determination of lead in iron salts use 10 ml of *ammonium citrate solution Sp.).* Add two drops of *phenol red solution* and make the solution just alkaline (red in colour) by the addition *of strong ammonnia solution.* Cool the solution if necessary, and add 2 ml of *potassium cyanide solution Sp.* Immediately extract the solution with several quantities each of 5 ml, of *dithizone extraction solution,* draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combine dithizone solutions for 30 seconds with 30 ml of a 1 per cent w/v solution of *nitric acid* and discrad the chloroform layer. Add to the solution exactly 5 ml of *standard dithizone solution* and 4 ml of *ammonia-cyanide solution Sp.* and shake for 30 seconds; the colour of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *dilute standard lead solution* equivalent to the amount of lead permitted in the sample under examination.

2.3.6. Limit Test for Sulphates

Reagents

Barium sulphate reagent – Mix 15 ml of 0.5 *M barium chloride,* 55 ml of *water,* and 20 ml of *sulphate free alcohol,* add 5 ml of a 0.0181 per cent w/v solution of potassium sulphate, dilute to 100 ml with *water,* and mix. Barium sulphate reagent

must be freshly prepared.

0.5 M Barium chloride – *Barium chloride* dissolved in *water* to contain in 1000 ml 122.1 g of $BaCl_2$, $2H_2O$.

Method

Dissolve the specified quantity of the substance in *water,* or prepare a solution as directed in the text, transfer to a *Nessler cylinder,* and add 2 ml of *dilute hydrochloric acid,* except where *hydrochloric acid* is used in the preparation of the solution. Dilute to 45 ml with *water*, add 5 ml of barium sulphate reagent. Stir immediately with a glass rod, and allow to stand for five minutes. The turbidity produced is not greater than the *standard turbidity*, when viewed transversely. Standard turbidity : Place 1.0 ml of 0.1089 per cent w/v solution of potassium sulphate and 2 ml of *dilute hydrochloric acid* in a *Nessler cylinder,* dilute to 45 ml with *water*, add 5 ml of barium sulphate reagent, stir immediately with a glass rod and allow to stand for five minutes.

2.3.7. ATOMIC ABSORPTION SPECTROPHOTOMETRY

Atomic absorption spectrophotometer is used in the determination of heavy metal elements and some nonmetal elements in the atomic state.

The light of characteristic wave length emitted from a cathodic discharge lamp is absorbed when it passes through the atomic vapor generated from sample containing the element being examined atomized to the ground state. The assay of the element being examined is tested by determing the decreased degree of light intensity of radiation. Atomic absorption obeys the general rule for absorption spectrophotometry. The assay is carried out by comparing the abosorbance of the test preparation with that of the reference preparation.

Apparatus

An atomic absorption spectrophotometer consists of a light source, an atomic generator, a monochromator and a detector system. Some are equipped with a background compensation system and automatic sampling system, etc.

1. **Light Source :** A hollow-cathode discharge lamp is usually used. The cathode is made of the element being examined.

2. **Atomic Generator :** There are four main types : flame atomizer, graphite furnace atomizer, hydride-generated atomizer and cold vapour atomiser.

   (1) **Flame atomizer** – It mainly consists of a nebulizer and a burner. Its function is to nebulize the test solution into aerosol, which is mixed with combustion gas and the mixture is introduced into the flame generated by the burner. So that the substance being examined is to be dried, evaporated to form the ground state atoms of the element being examined. The burning flame is generated by different mixtures of gases, acetylene-air is mostly used. By modifying the proportion of combustion gas, the temperature of the flame can be controlled and a better stability and a better sensitivity can be obtained.

(2) Furnace atomizer – It consists of electric furnace and a power supply. Its function is to dry and incinerate the substance being examined. During the stage of high temperature atomization, the ground state atoms of the element being examined are to be formed. Graphite is commonly used as the heater. Protection gas is introduced into the furnace to avoid oxidation and used to transfer the sample vapor.

(3) Hydride-generated atomizer – It consists of hydride generator and atomic absorption cell. It is used for the determination of the elements such as arsenic, selenium, stannum and antimony etc. Its function is to reduce the element to be examined in acidic medium to the low-boiling and easily pyrolyzed hydride. And then the hydride is swept by a stream of carrier gas into the atomic absorption cell which consists of quartz tube and heater etc., in which the hydride is pyrolyzed by heating to form the ground-state atom.

(4) Cold vapor atomizer – It consists of a mercury vapor atomizer and an absorption cell. It is suitable for the determination of mercury. Its function is to reduce the mercuric ion into mercury vapor which is swept into the quartz absorption cell by carrier gas.

3. Monochromator – Its function is to separate the specified wavelength radiation from the electromagnetic radiations eradiated from the light source. The optical path of the apparatus should assure the good spectra resolution and has the ability to work well at the condition of narrow spectral band (0.2 nm). The commonly used wavelength region is 190.0-900.0 nm.

4. Detector system – It consists of a detector, a signal processor and a recording system. It should have relatively higher sensitivity and better stability and can follow the rapid change of the signal absorption.

5. Background compensation system – System employed for the correction of atmospheric effects on the measuring system. Four principles can be utilized for background compensation : continuous spectrum sources (a deuterium lamp is often used in the UV region), the Zeeman effect, the self inversion phenomena and the non resonance spectrum. In the analysis using atomic absorption spectrophotometry, the interference to the determination caused by background and other reasons should be noticed. Changes of some experimental conditions, such as the wavelength, the slit width, the atomrizing condition, etc., may affect the sensitivity, the stability and the interference. If it is flame, the suitable wavelength, slit width and flame temperature, the addition of complexing agents and releasing agents and the use of Standard addition method may eliminate interference. If it is furnace, system, the selection of suitable background compensation system and the addition of suitable matrix modifying agents, etc may remove the interference. Background compensation method shall be selected as specified in the individual monograph.

Procedure

Method (direct calibration method) Prepare not less than 3 reference solutions of the element being examined of different concentrations, covering the range

recommended by the instrument manufacturer and add separately the corresponding reagents as that for the test solution and prepare the blank solution with the corresponding reagents. Measure the absorbances of the blank solution and each reference solution of different concentrations separately, record the readings and prepare a calibration curve with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa.

Prepare a test solution of the substance being examined as specified in the monograph, adjust the concentration to fall within the concentration range of the reference solution. Measure the absorbance 3 times, record the readings and calculate the average value. Interpolate the mean value of the readings on the calibration curve to determine the concentration of the element.

When used in the test for impurities, prepare two test preparations of the same concentration as specified in the monograph. To one of the test preparation add an amount of the reference substance equivalent to the limit of the element specified in the monograph. Proceed as directed above and measure this solution to give an appropriate reading a; then measure the test preparation without the addition of the reference substance under the same condition and record the reading b; b is not greater than (a-b).

Determination of Lead, Cadmium, Arsenic, Mercury and Copper.

(1) Determination of lead (graphite oven method).

I *Determination conditions* :- Reference condition: dry temperature: 100-120ºC, maintain 20 seconds; ash temperature: 400-750ºC, maintain 20-25 seconds; atomic temperature: 1700-2100ºC, maintain 4-5 seconds; measurement wavelength: 283.3 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

II *Preparation of lead standard stock solution* :- Measure accurately a quantity of lead single-element standard solution to prepare standard stock solution with 2% nitric acid solution, which containing 1 µg per ml, stored at 0-5ºC.

III *Preparation of calibration curve* :- Measure accurately a quantity of lead standard stock solutions respectively, diluted with 2% nitric acid solution to the concentration of 0, 5, 20, 40, 60, 80 ng per ml respectively. Measure respectively accurately 1 ml the above solution, add respectively 1 ml of 1% ammonium dihydrogen phosphate (ADP, $NH_4H_2PO_4$) and 0.2% magnesium nitrate [$Mg(NO_3)_2$], mix well, pipette accurately 20 µl to inject into the atomic generator of graphite oven and determine their absorbance, then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

IV *Preparation of test solution*

Method:- Weigh accurately about 0.5 g of the coarse powder of the substance being examined, transfer into a casparian flask, add 5-10 ml of the mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (4 : 1), add a small hopper

on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black in colour, add again a quantity of the above mixture, continuously heat till the solution becomes clear and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colourless and transparent or a little yellow, cool, transfer it into a 50 ml volumetric flask, wash the container with 2% nitric acid solution (HNO_3), add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

V *Determination* :- Measure accurately 1 ml of the test solution and its corresponding reagent blank solution respectively, add 1 ml of solution containing 1% $NH_4H_2PO_4$ and 0.2% Mg $(NO_3)_2$, shake well, pipette accurately 10-20 µl to determine their absorbance according to the above method of "Preparation of calibration curve". Calculate the content of lead (Pd) in the test solution from the calibration curve.

(2) Determination of cadmium (Cd) (graphite oven method).

I *Determination conditions* :- Reference condition: dry temperature: 100-120ºC, maintain 20 seconds; ash temperature: 300-500ºC, maintain 20-25 seconds; atomic temperature: 1500-1900ºC, maintain 4-5 seconds; measurement wavelength: 228.8 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

II *Preparation of Cd standard stock solution* :- Measure accurately a quantity of Cd single-element standard solution to prepare standard stock solution Cd with 2% HNO_3, which containing 0.4 µg per ml Cd, stored at 0-5ºC.

III *Preparation of calibration curve* :- Measure accurately a quantity of cadmium standard stock solutions, diluted to the concentration of 1.6, 3.2, 4.8, 6.4 and 8.0 ng per ml with 2% HNO_3 respectively. Pipette accurately 10 µl the above solutions respectively, inject them into the graphite oven, determine their absorbance, and then draw the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

IV *Preparation of test solution* :- Reference to method of "Preparation of test solution" of Pb in the above.

V *Determination* :- Pipette accurately 10-20 µl of the test solution and its corresponding reagent blank solution respectively, determine their absorbance according to the above method of "Preparation of calibration curve" (If interference occurs, weigh accurately respectively 1 ml of the standard solution, blank solution and test solution, add 1 ml of a solution containing 1% $NH_4H_2PO_4$ and 0.2% Mg $(NO_3)_2$, shake well, determine their absorbance according to the method above, calculate the content of Cd in the test solution from the calibration curve.

(3) Determination of arsenic (As) (hydride method).

I *Determination conditions* :- Apparatus suitable hydride generator device, reducing agent: a solution containing 1% sodium borohydride and 0.3% sodium hydroxide; carrier liquid: 1% hydrochloric acid; carrier gas: nitrogen; measurement wavelength: 193.7 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

II *Preparation of As standard stock solution* :- Measure accurately a quantity of As single-element standard solution to prepare standard stock solution with 2% nitric acid solution (HNO_3), which containing 1.0 µg per ml As, stored at 0-5ºC.

III *Preparation of calibration curve* :- Measure accurately proper quantity of arsenic standard stock solutions, diluted with 2% HNO_3 to the concentration of 2, 4, 8, 12 and 16 ng per ml respectively. Accurately transfer 10 ml of each into 25 ml volumetric flask respectively, add 1 ml of 25% potassium iodide solution (prepared prior to use), shake well, add 1 ml of ascorbic acid solution (prepared prior to use), shake well, dilute with hydrochloric acid solution (20-100) to the volume, shake well, close the stopper and immerse the flask in a water bath at 80ºC for 3 minutes. Cool, transfer proper quantities of each solution respectively into the hydride generator device, determine the absorbance, then plot the calibration curve with peak area (absorbance) as vertical axis and concentration as horizontal ordinate.

IV *Preparation of test solution* :- Reference to method of "Preparation of test solution" of Pb in the above.

V *Determination* :- Pipette accurately 10 ml of the test solution and its corresponding reagent blank solution respectively, proceed as described under "Preparation of calibration curve" beginning at the words "add 1 ml of 25% potassium iodide solution". Calculate the content of As in the test solution from the calibration curve.

(4) Determination of Mercury (Hg) (cold absorption method).

I *Determination conditions* :- Apparatus suitable hydride generator device; reducing agent: a solution containing 0.5% sodium borohydride and 0.1% sodium hydroxide; carrier liquid: 1% hydrochloric acid; carrier gas: nitrogen; measurement wavelength: 253.6 nm; background calibration: deuterium lamp (D lamp) or Zeeman effect.

II *Preparation of mercury standard stock solution* :- Measure accurately a proper quantity of mercury single-element standard solution to prepare standard stock solution with 2% nitric acid solution, which containing 1.0 µg per ml Hg, stored at 0-5ºC.

III *Preparation of calibration curve* :- Measure accurately 0, 0.1, 0.3, 0.5, 0.7 and 0.9 ml of mercury standard stock solution, transfer into a 50 ml volumetric flask respectively, add 40 ml 4% sulfuric acid solution and 0.5 ml of 5% potassium permanganate solution, shake well, drop 5% hydroxylamine

hydrochloride solution until the violet red just disappears, dilute with 4% sulfuric acid solution to the volume, shake well. A quantity of each solution is injected to the hydride generator device, determine the absorbance, then plot the calibration curve with peak area (absorbance) as vertical axis and concentration as horizontal ordinate.

IV *Preparation of test solution*

Method :- Transfer 1 g of the coarse powder of the substance being examined, accurately weighed, into a casparian flask, add 5-10 ml of the mixture solution of nitric acid solution (HNO_3) and perchloric acid ($HClO_4$) (4 : 1), mix well, fix a small hopper on the flask-top, immerse overnight, heat to slake on the electric hot plate at 120-140ºC for 4-8 hours until slaking completely, cool, add a quantity of 4% sulfuric acid solution and 0.5 ml of 5% potassium permanganate solution, shake well, drop 5% hydroxylamine hydrochloride solution until the violet red colour just disappears, dilute with 4% H_2SO_4 solution to 25 ml, shake well, centrifugate if necessary, the supernatant is used as the test solution. Prepare synchronally the reagent blank solution based on the same procedure.

V *Determination* :- Pipette accurately a quantity of the test solution and its corresponding reagent blank solution, respectively, proceed as described under "Preparation of calibration curve" beginning at the words "add 1 ml of 25% potassium iodide solution". Calculate the content of mercury (Hg) in the test solution from the calibration curve.

(5) Determination of Copper (flame method).

I *Determination conditions* :- Measurement wavelength: 324.7 nm; flame: air -acetylene flame; background calibration: deuterium lamp or Zeeman effect.

II *Preparation of copper standard stock solution* :- Measure accurately a proper quantity of copper single-element standard solution, to prepare the standard stock solution with 2% nitric acid solution, which containing 10 µg per ml Cu, stored at 0-5ºC.

III *Preparation of calibration curve* :- Measure accurately a quantity of copper standard stock solutions, dilute with 2% HNO_3 to the concentrations of 0.05, 0.2, 0.4, 0.6 and 0.8 µg per ml respectively. Inject each standard solution into the flame and determine the absorbance, respective, then plot the calibration curve with absorbance as vertical axis and concentration as horizontal ordinate.

IV *Preparation of test solution* :- Reference to method of "Preparation of test solution" of Pb in the above.

V *Determination* :- Pipette accurately quantities of the test solution and its corresponding reagent blank solution respectively, proceed as described under "Preparation of calibration curve". Calculate the content of Cu in the test solution from the calibration curve.

OR

2.3.8. INDUCTIVELY-COUPLED PLASMA MASS SPECTROMETRY.

This method is provided to determine arsenic, cadmium, lead, mercury and copper in Traditional Herbal Medicinal substances using inductively-coupled plasma mass spectrometer. The instrument consists of plasma ionization and quadrupole mass spectrometer. The plasma ionization is made up of sampling system, atomization device, atomizer chamber, quartz fire-cannulation and sampling cone; mass spectrometers is made up of quadrupoles analyzer, detector and other components.

(1) *Preparation of reference stock solution* :- Measure accurately a quantity of arsenic, cadmium, lead and mercury single-element standard solution, into a volumetric flask respectively and prepare respective stock solution of 1 µg per ml lead, 0.5 µg per ml arsenic, 1 µg per ml cadmium, 1 µg per ml mercury and 10 µg per ml copper with 10% nitric acid solution (HNO_3).

(2) *Preparation of reference solution* :- Measure accurately a quantity of reference stock solutions of lead, arsenic, cadmium and copper to prepare the mixture solution containing arsenic and lead with respective concentration of 0, 1, 5, 10 and 20 ng per ml, cadmium with the concentration of 0, 0.5, 2.5, 5 and 10 ng per ml and copper with the concentration of 0, 50, 100, 200, 500 ng per ml with 10% HNO_3 solution. Measure accurately a quantity of mercury reference stock solution, diluted with 10% HNO_3 to the concentrations of 0, 0.2, 0.5, 1, 2 and 5 ng per ml. This solution should be prepared prior to use.

(3) *Preparation of internal standard solution* :- Measure precisely a quantity of single standard solution of germanium (Ge) indium (In) and bismuth (Bi), prepare the mixture solution with their respective concentration of 1 µg per ml.

(4) *Preparation of test solution* :- Substances being examined are dried for 2 hours at 60ºC, ground to coarse powder, weighed accurately 0.5 g of each and put into the microwave slaking tank with the ability of pressurization and resistibility to elevated temperature, add 5-10 ml of HNO_3 (given severe reaction, lay aside till the reaction ceases). Close tightly and slake according to the instrumental requirements and relative procedures. After slaking completely, cool to the temperature of slaked solution lower than 60ºC, take out the slaking-tank, cool, transfer the slaked solution into 50 ml volumetric flask, wash the slaking-tank with a little water three times and add them into the same flask, add 200 µl of 1 µg/ml aurum single-element standard solution, dilute to the volume, shake well (if there is some deposit, get the supernatant after centrifugation).

Prepare the reagent blank solution according to the same procedure except not adding aurum single-element standard solution.

(5) *Determination* :- The chosen isotopes to detect are ^{63}Cu, ^{75}As, ^{114}Cd, ^{202}Hg and ^{208}Pb. Select ^{72}Ge as the internal standard of ^{63}Cu, ^{75}As, ^{115}In as the internal standard of ^{114}Cd, ^{209}Bi as the internal standard of ^{202}Hg and ^{208}Pb and correct the detected elements by choosing the suitable correction equation according to the requirements of different instruments.

Insert the internal standard sampling pipe of instrument in the internal standard solution during the analyzing, while insert the sampling pipe in turn into the different concentrations control solutions and detect. Plot the calibration curve with the detected values (mean value of three readings) as vertical axis and concentrations as horizontal ordinate. Insert the sampling pipe into the test solution, detect and get the average value of three readings. Calculate the corresponding concentrations from the calibration curve, subtract the concentrations of the corresponding blank solutions and then obtain the content of the above elements respectively.

2.4.1. MICROBIAL LIMIT TESTS

The following tests are designed for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. The term 'growth' is used to designate the presence and presumed proliferation of viable micro-organisms.

Preliminary Testing

The methods given herein are invalid unless it is demonstrated that the test specimens to which they are applied do not, of themselves, inhibit the multiplication under the test conditions of micro-organisms that can be present. Therefore, prior to doing the tests, inoculate diluted specimens of the substance being examined with separate viable cultures of Escherichia coli, Salmonella species, Pseudomonas aeruginosa and Staphylococcus aureus. This is done by adding 1 ml of not less than 10^{-3} dilutions of a 24-hr broth culture of the micro-organisms to the first dilution (in buffer solution pH 7.2, fluid soyabean-casein digest medium or fluid lactose medium) of the test material and following the test procedure. If the organisms fail to grow in the relevant medium the procedure should be modified by (a) increasing the volume of diluent with the quantity of test material remaining the same, or (b) incorporating a sufficient quantity of a suitable inactivating agent in the diluents, or (c) combining the aforementioned modifications so as to permit growth of the organisms in the media. If inhibitory substances are present in the sample, 0.5% of soya lecithin and 4% of polysorbate 20 may be added to the culture medium. Alternatively, repeat the test as described in the previous paragraph, using fluid casein digest-soya lecithin-polysorbate 20 medium to demonstrate neutralization of preservatives or other antimicrobial agents in the test material. Where inhibitory substances are contained in the product and the latter is soluble, the Membrane filtration method described under Total Aerobic Microbial Count may be used.

If inspite of incorporation of suitable inactivating agents and a substantial increase in the volume of diluent it is still not possible to recover the viable cultures described above and where the article is not suitable for applying the membrance filtration method, it can be assumed that the failure to isolate the inoculated organism may be due to the bactericidal activity of the product. This may indicate that the article is not likely to be contaminated with the given species of micro-organisms. However, monitoring should be continued to establish the spectrum of inhibition and bactericidal activity of the article.

Media

Culture media may be prepared as given below or dehydrated culture media may be used provided that, when reconstituted as directed by the manufacturer, they have similar ingredients and / or yield media comparable to those obtained from the formulae given below.

Where agar is specified in a formula, use agar that has a moisture content of not more than 15%. Where water is called for in a formula, use purified water. Unless otherwise indicated, the media should be sterilized by heating in an autoclave at 115° for 30 minutes.

In preparing media by the formulas given below, dissolve the soluble solids in the water, using heat if necessary, to effect complete solution and add solutions of hydrochloric acid or sodium hydroxide in quantities sufficient to yield the required pH in the medium when it is ready for use. Determine the pH at 25º ± 2º.

Baird-Parker Agar Medium

Pancreatic digest of casein	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Agar	20.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Water to	1000 ml

Heat with frequent agitation and boil for 1 minute. Sterilise, cool to between 45º and 50º, and add 10 ml of a 1% w/v solution of sterile potassium tellurite and 50 ml of egg-yolk emulsion. Mix intimately but gently and pour into plates. (Prepare the egg-yolk emulsion by disinfecting the surface of whole shell eggs, aseptically cracking the eggs, and separating out intact yolks into a sterile graduated cylinder. Add sterile saline solution, get a 3 to 7 ratio of egg-yolk to saline. Add to a sterile blender cup, and mix at high speed for 5 seconds). Adjust the pH after sterilization to 6.8 ± 0.2.

Bismuth Sulphite Agar Medium

Solution (1)	
Beef extract	6 g
Peptone	10 g
Agar	24 g
Ferric citrate	0.4 g
Brilliant green	10 mg
Water to	1000 ml

Dissolve with the aid of heat and sterilise by maintaining at 115º for 30 minutes.

Solution (2)	
Ammonium bismuth citrate	3 g
Sodium sulphite	10 g
Anhydrous disodium hydrogen phosphate	5 g
Dextrose monohydrate	5 g
Water to	100 ml

Mix, heat to boiling, cool to room temperature, add 1 volume of solution (2) to 10 volumes of solution (1) previously melted and cooled to a temperature of 55º and pour.

Bismuth Sulphite Agar Medium should be stored at 2º to 8º for 5 days before use.

Brilliant Green Agar Medium

Peptone	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Phenol red	80.0 g
Brilliant green	12.5 mg
Agar	12.0 g
Water to	1000 ml

Mix, allow to stand for 15 minutes, sterilise by maintaining at 115º for 30 minutes and mix before pouring.

Buffered Sodium Chloride-Peptone Solution pH 7.0

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate	7.23 g
Sodium chloride	4.30 g
Peptone (meat or casein)	1.0 g
Water to	1000 ml

0.1 to 1.0% w/v polysorbate 20 or polysorbate 80 may be added. Sterilise by heating in an autoclave at 121º for 15 minutes.

Casein Soyabean Digest Agar Medium

Pancreatic digest of casein	15.0 g
Papaic digest of soyabean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water to	1000 ml

Adjust the pH after sterilization to 7.3±0.2.

Cetrimide Agar Medium

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Glycerin	10.0 g
Water to	1000 ml

Heat to boiling for 1 minute with shaking. Adjust the pH so that after sterilization it is 7.0 to 7.4. Sterilise at 121° for 15 minutes.

Desoxycholate-Citrate Agar Medium

Beef extract	5.0 g
Peptone	5.0 g
Lactose	10.0 g
Trisodium citrate	8.5 g
Sodium thiosulphate	5.4 g
Ferric citrate	1.0 g
Sodium desoxycholate	5.0 g
Neutral red	0.02 g
Agar	12.0 g
Water to	1000 ml

Mix and allow to stand for 15 minutes. With continuous stirring, bring gently to the boil and maintain at boiling point until solution is complete. Cool to 80°, mix, pour and cool rapidly.

Care should be taken not to overheat Desoxycholate Citrate Agar during preparation. It should not be remelted and the surface of the plates should be dried before use.

Fluid Casein Digest-Soya Lecithin-Polysorbate 20 Medium

Pancreatic digest of casein	20 g
Soya lecithin	5 g
Polysorbate 20	40 ml
Water to	1000 ml

Dissolve the pancreatic digest of casein and soya lecithin in water, heating in a water-bath at 48º to 50º for about 30 minutes to effect solution. Add polysorbate 20, mix and dispense as desired.

Fluid Lactose Medium

Beef extract	3.0 g
Pancreatic digest of gelatin	5.0 g
Lactose	5.0 g
Water to	1000 ml

Cool as quickly as possible after sterilization. Adjust the pH after sterilization to 6.9±0.2.

Lactose Broth Medium

Beef extract	3.0 g
Pancreatic digest of gelatin	5.0 g
Lactose	5.0 g
Water to	1000 ml

Adjust the pH after sterilisation to 6.9±0.2.

Levine Eosin-Methylene Blue Agar Medium

Pancreatic digest of gelatin	10.0 g
Dibasic potassium phosphate	2.0 g
Agar	15.0 g
Lactose	10.0 g
Eosin Y	400 mg
Methylene blue	65 mg
Water to	1000 ml

Dissolve the pancreatic digest of gelatin, dibasic potassium phosphate and agar in water with warming and allow to cool. Just prior to use, liquefy the gelled agar solution and the remaining ingredients, as solutions, in the following amounts and mix. For each 100 ml of the liquefied agar solution use 5 ml of a 20% w/v solution of lactose, and 2 ml of a 2% w/v solution of eosin Y, and 2 ml of a 0.33% w/v solution of methylene blue. The finished medium may not be clear. Adjust the pH after sterilisation to 7.1±0.2.

MacConkey Agar Medium

Pancreatic digest of gelatin	17.0 g
Peptone (meat and casein, equal parts)	3.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30 mg
Crystal violet	1 mg
Water to	1000 ml

Boil the mixture of solids and water for 1 minute to effect solution. Adjust the pH after sterilisation to 7.1±0.2.

MacConkey Broth Medium

Pancreatic digest of gelatin	20.0 g
Lactose	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Water to	1000 ml

Adjust the pH after sterilisation to 7.3±0.2.

Mannitol-Salt Agar Medium

Pancreatic digest of gelatin	5.0 g
Peptic digest of animal tissue	5.0 g
Beef extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25 mg
Water to	1000 ml

Mix, heat with frequent agitation and boil for 1 minute to effect solution. Adjust the pH after sterilisation to 7.4±0.2.

Nutrient Agar Medium : Nutrient broth gelled by the addition of 1 to 2% w/v of agar.

Nutrient Broth Medium

Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5 mg
Water to	1000 ml

Dissolve with the aid of heat. Adjust the pH to 8.0 to 8.4 with 5M sodium hydroxide and boil for 10 minutes. Filter, and sterilise by maintaining at 115º for 30 minutes and adjust the pH to 7.3±0.1.

Pseudomonas Agar Medium for Detection of Flourescein

Pancreatic digest of casein	10.0 g
Peptic digest of animal tissue	10.0 g
Anhydrous dibasic potassium phosphate	1.5 g
Magnesium sulphate ($MgSO_4$, $7H_2O$)	1.5 g
Glycerin	10.0 ml
Agar	15.0 g
Water to	1000 ml

Dissolve the solid components in water before adding glycerin. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the pH after sterilisation to 7.2±0.2.

Pseudomonas Agar Medium for Detection of Pyocyanin

Pancreatic digest of gelatin	20.0 g
Anhydrous magnesium chloride	1.4 g
Anhydrous potassium sulphate	10.0 g
Agar	15.0 g
Glycerin	10.0 ml
Water to	1000 ml

Dissolve the solid components in water before adding glycerin. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the pH after sterilisation to 7.2±0.2.

Sabouraud Dextrose Agar Medium

Dextrose	40 g
Mixture of equal parts of peptic digest of animal tissue and pancreatic digest of casein	10 g
Agar	15 g
Water to	1000 ml

Mix, and boil to effect solution. Adjust the pH after sterilisation to 5.6±0.2.

Sabouraud Dextrose Agar Medium with Antibiotics

To 1 liter of Sabouraud Dextrose Agar Medium add 0.1 g of benzylpenicillin sodium and 0.1 g of tetracycline or alternatively add 50 mg of chloramphenicol immediately before use.

Selenite F Broth

Peptone	5 g
Lactose	4 g
Disodium hydrogen phosphate	10 g
Sodium hydrogen selenite	4 g
Water to	1000 ml

Dissolve, distribute in sterile containers and sterilise by maintaining at 100° for 30 minutes.

Fluid Selenite-Cystine Medium

Pancreatic digest of casein	5.0 g
Lactose	4.0 g
Sodium phosphate	10.0 g
Sodium hydrogen selenite	4.0 g
L-Cystine	10.0 mg
Water to	1000 ml

Mix and heat to effect solution. Heat in flowing steam for 15 minutes. Adjust the final pH to 7.0±0.2. Do not sterilise.

Fluid Soyabean-Casein Digest Medium

Prepare as directed under tests for sterility, Appendix III.

Tetrathionate Broth Medium

Beef extract	0.9 g
Peptone	4.5 g
Yeast extract	1.8 g
Sodium chloride	4.5 g
Calcium carbonate	25.0 g
Sodium thiosulphate	40.7 g
Water to	1000 ml

Dissolve the solids in water and heat the solution to boil. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 ml of water.

Tetrathionate-Bile-Brilliant Green Broth Medium

Peptone	8.6 g
Dehydrated ox bile	8.0 g
Sodium chloride	6.4 g
Calcium carbonate	20.0 g
Potassium tetrathionate	20.0 g
Brilliant green	70 mg
Water to	1000 ml

Heat just to boiling; do not reheat. Adjust the pH so that after heating it is 7.0±0.2.

Triple Sugar-Iron Agar Medium

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose monohydrate	1.0 g
Ferrous sulphate	0.2 g
Sodium chloride	5.0 g
Sodium thiosulphate	0.3 g
Phenol red	24 mg
Agar	12.0 g
Water to	1000 ml

Mix, allow standing for 15 minutes, bringing to boil and maintain at boiling point until solution is complete, mix, distributing in tubes and sterilising by maintaining at 115 º for 30 minutes. Allow to stand in a sloped form with a butt about 2.5 cm long.

Urea Broth Medium

Potassium dihydrogen orthophosphate	9.1 g
Anhydrous disodium hydrogen phosphate	9.5 g
Urea	20.0 g
Yeast extract	0.1 g
Phenol red	10 mg
Water to	1000 ml

Mix, sterilise by filtration and distribute aseptically in sterile containers.

Vogel-Johnson Agar Medium

Pancreatic digest of casein	10.0 g
Yeast extract	5.0 g
Mannitol	10.0 g
Dibasic potassium phosphate	5.0 g
Lithium chloride	5.0 g
Glycerin	10.0 g
Agar	16.0 g
Phenol red	25.0 mg
Water to	1000 ml

Boil the solution of solids for 1 minute. Sterilise, cool to between 45º to 50º and add 20 ml of a 1% w/v sterile solution of potassium tellurite. Adjust the pH after sterilisation to 7.0±0.2.

Xylose-Lysine-Desoxycholate Agar Medium

Xylose	3.5 g
L-Lysine	5.0 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g

Phenol red	80 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	800 mg
Water to	1000 ml

Heat the mixture of solids and water, with swirling, just to the boiling point. Do not overheat or sterilise. Transfer at once to a water-bath maintained at about 50º and pour into plates as soon as the medium has cooled. Adjust the final pH to 7.4±0.2.

Sampling : Use 10 ml or 10 g specimens for each of the tests specified in the individual monograph.

Precautions : The microbial limit tests should be carried out under conditions designed to avoid accidental contamination during the test. The precautions taken to avoid contamination must be such that they do not adversely affect any micro-organisms that should be revealed in the test.

Sampling : Use 10 ml or 10 g specimens for each of the tests specified in the individual monograph.

Precautions : The microbial limit tests should be carried out under conditions designed to avoid accidental contamination during the test. The precautions taken to avoid contamination must be such that they do not adversely affect any micro-organisms that should be revealed in the test.

Methods

2.4.2. TOTAL AEROBIC MICROBIAL COUNT

Pretreat the sample of the product being examined as described below.

Water soluble products : Dissolve 10 g or dilute 10 ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution pH 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the pH to about 7.

Products insoluble in water (non-fatty) : Suspend 10 g or 10 ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution pH 7.0 or any other suitable medium shown not to have antimicrobial activity under the conditions of the test and dilute to 100 ml with the same medium. If necessary, divide the preparation being examined and homogenize the suspension mechanically.

A suitable surface-active agent such as 0.1% w/v of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary, adjust the pH of the suspension to about 7.

Fatty products : Homogenise 10 g or 10 ml of the preparation being examined, unless otherwise specified, with 5 g of polysorbate 20 or polysorbate 80. If necessary, heat to not more than 40º. Mix carefully while maintaining the temperature in the water-bath or in an oven. Add 85 ml of buffered sodium chloride-peptone solution pH 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of the test, heated to not more than 40º if necessary. Maintain this temperature for the shortest time necessary for formation of an emulsion and in any case for not more than 30 minutes. If necessary, adjust the pH to about 7.

Examination of the sample

Determine the total aerobic microbial count in the substance being examined by any of the following methods.

Membrane filtration : Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined. Sterilise and assemble the filtration apparatus described under *tests for sterility,* Appendix- III.

Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as *buffered sodium chloride-peptone solution pH 7.0.* For fatty substances add to the liquid *polysorbate 20* or *polysorbate 80.* Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of *casein soyabean digest agar* and the other, intended for the enumeration of fungi, to the surface of a plate of *Sabouraud dextrose agar with antibiotics.*

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30º to 35º in the test for bacteria and 20º to 25º in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per g or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.

Plate count: *For bacteria* – Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied *casein soyabean digest agar* at not more than 45º. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30º to 35º for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

For fungi – Proceed as described in the test for bacteria but use *Sabouraud dextrose agar with antibiotics* in place of *casein soyabean digest agar* and incubate the plates at 20º to 25º for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

Multiple-tube or serial dilution method : In each of fourteen test-tubes of similar size place 9.0 ml of sterile *fluid soyabean casein digest medium.* Arrange twelve of the tubes in four sets of three tubes each. Put aside one set of three tubes to serve as controls. Into each of three tubes of one set ("100") and into fourth tube (A) pipette 1 ml of the solution of suspension of the test specimen and mix. From tube A pipette 1 ml of its contents into the one remaining tube (B) not included in the set and mix. These two tubes contain 100 mg (or 100 µl)and 10 mg (or 10 µl) of the specimen respectively. Into each of the second set ("10") of three tubes pipette 1 ml from tube

A, and into each tube of the third set ("1") pipette 1 ml from tube B. Discard the unused contents of tube A and B. Close well and incubate all of the tubes. Following the incubation period, examine the tubes for growth. The three control tubes remain clear. Observations in the tubes containing the test specimen, when interpreted by reference to Table 1, indicate the most probable number of micro-organisms per g or per ml of the test specimen.

Table 1 – Most probable total count by multiple-tube or serial dilution method

Observed combination of numbers of tubes showing growth in each set			*Most probable number of micro-organisms per g or per ml*
No.of mg (or ml) of specimen per tube			
100(100 µl)	10(10 µl)	1(1 µl)	
3333	3333	3210	>11001100500200
3333	2222	3210	29021015090
3333	1111	3210	1601207040
3333	0000	3210	95604023

2.4.3. TESTS FOR SPECIFIED MICRO-ORGANISMS

Pretreatment of the sample being examined – Proceed as described under the test for total aerobic microbial count but using lactose broth or any other suitable medium shown to have no antimicrobial activity under the conditions of test in place of buffered sodium chloride-peptone solution pH 7.0.

Escherichia coli : Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow to stand for 1 hour (4 hours for gelatin) and shake again. Loosen the cap and incubate at 37º for 18 to 24 hours.

Primary test – Add 1.0 ml of the enrichment culture to a tube containing 5 ml of MacConkey broth. Incubate in a water-bath at 36º to 38º for 48 hours. If the contents of the tube show acid and gas carry out the secondary test.

Secondary test – Add 0.1 ml of the contents of the tubes containing (a) 5 ml of MacConkey broth, and (b) 5 ml of peptone water. Incubate in a water-bath at 43.5º to 44.5º for 24 hours and examine tube (a) for acid and gas and tube (b) for indole. To

test for indole, add 0.5 ml of Kovac's reagent, shake well and allow to stand for 1 minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of Escherichia coli.

Carry out a control test by repeating the primary and secondary tests adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Escherichia coli (NCTC 9002) organisms, prepared from a 24-hour culture in nutrient broth, to 5 ml of MacConkey broth. The test is not valid unless the results indicate that the control contains Escherichia coli.

Alternative test – By means of an inoculating loop, streak a portion from the enrichment culture (obtained in the previous test) on the surface of MacConkey agar medium. Cover and invert the dishes and incubate. Upon examination, if none of the colonies are brick-red in colour and have a surrounding zone of precipitated bile the sample meets the requirements of the test for the absence of Escherichia coli.

If the colonies described above are found, transfer the suspect colonies individually to the surface of Levine eosin-methylene blue agar medium, plated on Petri dishes. Cover and invert the plates and incubate. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, the sample meets the requirements of the test for the absence of Escherichia coli. The presence of Escherichia coli may be confirmed by further suitable cultural and biochemical tests.

Salmonella : Transfer a quantity of the pretreated preparation being examined containing 1 g or 1 ml of the product to 100 ml of nutrient broth in a sterile screw-capped jar, shake, allow to stand for 4 hours and shake again. Loosen the cap and incubate at 35º to 37º for 24 hours.

Primary test – Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36º to 38º for 48 hours. From each of these two cultures subculture on at least two of the following four agar media: bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose-lysine-desoxycholate agar. Incubate the plates at 36º to 38º for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 2, the sample meets the requirements of the test for the absence of the genus Salmonella.

If any colonies conforming to the description in Table 2 are produced, carry out the secondary test.

Secondary test – Subculture any colonies showing the characteristics given in Table 2 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle, and at the same time inoculate a tube of urea broth. Incubate at 36º to 38º for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in the urea broth, indicates the presence of salmonellae. If acid but no gas is produced in the sub culture, the identity of the organisms should be confirmed by agglutination tests.

Table 2 – Test for Salmonella

Medium	*Description of colony*
Bismuth sulphite agar	Black or green
Brilliant green agar	Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate-citrate agar	Colourless and opaque, with or without black centers
Xylose-lysine-desoxy-cholate agar	Red with or without black centres

Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Salmonella abony (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains Salmonella.

Pseudomonas aeruginosa : Pretreat the preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35º to 37º for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium on the surface of cetrimide agar medium, each plated on Petri dishes. Cover and incubate at 35º to 37º for 18 to 24 hours.

If, upon examination, none of the plates contains colonies having the characteristics listed in Table 3 for the media used, the sample meets the requirement for freedom from Pseudomonas aeruginosa. If any colonies conforming to the description in Table 3 are produced, carry out the oxidase and pigment tests.

Streak representative suspect colonies from the agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluorescein and pseudomonas agar medium for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media and incubate at 33º to 37º for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table 3 are present.

If growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1% w/v solution of N,N,N^1,N^1-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink colour, changing to purple, the sample meets the requirements of the test for the absence of Pseudomonas aeruginosa.

Staphylococcus aureus : Proceed as described under **Pseudomonas aeruginosa.** If, upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table 4 for the media used, the sample meets the requirements for the absence of Staphylococcus aureus.

Table 3 – Tests for Pseudomonas aeruginosa

Medium	*Characteristic colonial morphology*	*Fluorescence in UV light*	*Oxidase test*	*Gram stain*
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for detection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

Table 4 – Tests for Staphylococcus aureus

Selective medium	*Characteristic colonial morphology*	*Gram stain*
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)
Mannitol-salt agar	Yellow colonies with yellow zones	Positive cocci (in clusters)
Baird-Parker agar	Black, shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table 4 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives. Incubate in water-bath at 37º examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of Staphylococcus aureus.

Validity of the tests : For total aerobic microbial count – Grow the following test strains separately in tubes containing fluid soyabean-casein digest medium at 30º to 35º for 18 to 24 hours or, for Candida albicans, at 20º for 48 hours.

Staphylococcus aureus	(ATCC 6538; NCTC 10788)
Bacillus subtilis	(ATCC 6633; NCIB 8054)
Escherichia coli	(ATCC 8739; NCIB 8545)
Candida albicans	(ATCC 2091; ATCC 10231)

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 100 viable micro-organisms per ml. Use the suspension of each of the micro-organisms separately as a control of the counting methods, in the presence and absence of the preparation being examined, if necessary.

A count for any of the test organisms differing by not more than a factor of 10 from the calculated value for the inoculum should be obtained. To test the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total aerobic microbial count method using sterile buffered sodium chloride-peptone solution pH 7.0 as the test preparation. There should be no growth of micro-organisms.

For specified micro-organisms –Grow separately the test strains of Staphylococcus aureus and Pseudomonas aeruginosa in fluid soyabean-casein digest medium and Escherichia coli and Salmonella typhimurium at 30° to 35° for 18 to 24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 10^3 viable micro-organisms per ml. Mix equal volume of each suspension and use 0.4 ml (approximately 10^2 micro-organisms of each strain) as an inoculum in the test for E. coli, salmonellae, P. aeruginosa and S. aureus, in the presence and absence of the preparation being examined, if necessary. A positive result for the respective strain of micro-organism should be obtained.

2.5.1. PESTICIDE RESIDUES

Definition. For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing storage, transport or marketing of vegetable drugs. The item includes substances intended for use as growth-regulators, defoliants or desiccants and any substance applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport.

Limits. Unless otherwise indicated in the monograph, the drug to be examined at least complies with the limits indicated in Table -1, The limits applying to pesticides that are not listed in the table and whose presence is suspected for any reason comply with the limits set by European Community directives 76/895 and 90/642, including their annexes and successive updates. Limits for pesticides that are not listed in Table.-1 nor in EC directives are calculated using the following expression :

$$\frac{ADI \times M}{MDD \times 100}$$

ADI = acceptable daily intake, as published by FAO-WHO, in milligrams per kilogram of body mass,

M = body mass in kilograms (60 kg),

MDD = daily dose of the drug, in kilograms.

If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$\frac{ADI \times M \times E}{MDD \times 100}$$

E = extraction factor of the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely.

SAMPLING

Method. For containers up to 1 kg, take one sample from the total content, thoroughly mixed, sufficient for the tests. For containers between 1 kg and 5 kg, take

three samples, equal in volume, from the upper, middle and lower parts of the container, each being sufficient to carry out the tests. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests. For containers of more than 5 kg, take three samples, each of at least 250 g from the upper, middle and lower parts of the container. Thoroughly mix the samples and take from the mixture an amount sufficient to carry out the tests.

Size of sampling. If the number (n) of containers is three or fewer, take samples from each container as indicated above under Method. If the number of containers is more than three, take n+1 samples for containers as indicated under Method, rounding up to the nearest unit if necessary.

The samples are to be analysed immediately to avoid possible degradation of the residues. If this is not possible, the samples are stored in airtight containers suitable for food contact, at a temperature below 0ºC, protected from light.

Reagents. All reagents and solvents are free from any contaminants, especially pesticides, that might interfere with the analysis. It is often necessary to use special quality solvents or, if this is not possible, solvents that have recently been re-distilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

Apparatus. Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 h in a solution of phosphate-free detergent, rinse with large quantities of *distilled water R* and wash with acetone and hexane or heptane.

Qualitative and quantitative analysis of pesticide residues.

The analytical procedures used are validated according to the regulations in force. In particular, they satisfy the following criteria :

- the chosen method, especially the purification steps, are suitable for the combination pesticide residue/substance to be analysed and not susceptible to interference from co-extractives; the limits of detection and quantification are measured for each pesticide-matrix combination to be analysed.
- between 70 per cent to 110 per cent of each pesticide is recovered.
- the repeatability of the method is not less than the values indicated in Table 2.8.
- the reproducibility of the method is not less than the values indicated in Table 2.8.
- the concentration of test and reference solutions and the setting of the apparatus are such that a linear response is obtained from the analytical detector.

Table - 1

Substance	*Limit (mg/kg)*
Alachlor	0.02
Aldrin and Dieldrin (sum of)	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of cis-, trans – and Oxythlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of p,p-'DDT, o,p-'DDT, p,p-'DDE and p,p-'TDE	1.0
Deltamethrin	0.5
Diazinon	0.5
Dichlorvos	1.0
Dithiocarbamates (as CS_2)	2.0
Endosulfan (sum of isomers and Endosulfan sulphate)	3.0
Endrin	0.05
Ethion	2.0
Fenitrothion	0.5
Fenvalerate	1.5
Fonofos	0.05
Heptachlor (sum of Heptachlor and Heptachlorepoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than ã)	0.3
Lindane (ã-Hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.2
Permethrin	1.0
Phosalone	0.1
Piperonyl butoxide	3.0
Pirimiphos-methyl	4.0
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0

Table -2

Concentration of the pesticide (mg/kg)	*Repeatability (difference, ± mg/kg)*	*Reproducibility (difference, ± mg/kg)*
0.010	0.005	0.01
0.100	0.025	0.05
1.000	0.125	0.25

The following section is published for information.

2.5.2. Test for pesticides

ORGANOCHLORINE, ORGANOPHOSPHORUS AND PYRETHROID INSECTICIDES.

The following methods may be used, in connection with the general method above, depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry...) or a different method (immunochemical methods..) to confirm the results obtained.

This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 per cent of water. Samples with a higher content of water may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

1. EXTRACTION

To 10 g of the substance being examined, coarsely powdered, add 100 ml of *acetone R* and allow to stand for 20 mim. Add 1 ml of a solution containing 1.8 µg/ml of *carbophenothion R* in *toluene R.* Homogenise using a high-speed blender for 3 min. Filter and wash the filter cake with two quantities, each of 25 ml, of *acetone R.* Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of *toluene R* and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of *toluene R.* Filter through a membrane filter (45 µm), rinse the flask and the filter with *toluene R* and dilute to 10.0 ml with the same solvent (solution A).

2. PURIFICATION

2.1. Organochlorine, organophosphorus and pyrethroid insecticides.

Examine by size-exclusion chromatography.

The chromatographic procedure may be carried out using:

- a stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with *styrene-divinylbenzene copolymer R* (5 µm).

– as mobile phase *toluene R* at a flow rate of 1 ml/min.

Performance of the column. Inject 100 µl of a solution containing 0.5 g/l of *methyl red R* and 0.5 g/l of *oracet blue 2R R* in *toluene R* and proceed with the chromatography. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, using a solution containing, in *toluene R,* at a suitable concentration, the insecticide to be analysed with the lowest molecular mass (for example, dichlorvos) and that with the highest molecular mass (for example, deltamethrin). Determine which fraction of the eluate contains both insecticides.

Purification of the test solution. Inject a suitable volume of solution A (100 µl to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

2.2. Organochlorine and pyrethroid insecticides.

In a chromatography column, 0.10 m long and 5 mm in internal diameter, introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows: heat *silica gel for chromatography R* in an oven at 150ºC for at least 4 h. Allow to cool and add dropwise a quantity of *water R* corresponding to 1.5 per cent of the mass of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 h using a mechanical shaker. Condition the column using 1.5 ml of *hexane R.* Prepacked columns containing about 0.50 g of a suitable silica gel may also be used provided they are previously validated.

Concentrate solution B in a current of *helium for chromatography R* or *oxygen-free nitrogen R* almost to dryness and dilute to a suitable volume with *toluene R* (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed with the chromatography using 1.8 ml of *toluene R* as the mobile phase. Collect the eluate (solution C).

2.5.3. QUANTITATIVE ANALYSIS

3.1. Organophosphorus insecticides.

Examine by gas chromatography 2.28), using *carbophenothion R* as internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Test solution. Concentrate solution B in a current of *helium for chromatography R* almost to dryness and dilute to 100 µl with *toluene R.*

Reference solution. Prepare at least three solutions in *toluene R* containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

The chromatographic procedure may be carried out using :

- a fused-silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of *poly (dimethyl) siloxane R.*
- *hydrogen for chromatography R* as the carrier gas. Other gases such as helium for *chromatography R* or *nitrogen for chromatography R* may also be used provided the chromatography is suitably validated.
- a phosphorus-nitrogen flame-ionisation detector or a atomic emission spectrometry detector.

Maintaining the temperature of the column at 80ºC for 1 min, then raising it at a rate of 30ºC/min to 150ºC, maintaining at 150ºC for 3 min, then raising the temperature at a rate of 4ºC/min to 280ºC and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250ºC and that of the detector at 275ºC. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table-3. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

3.2. Organochlorine and pyrethroid insecticides.

Examine by gas chromatography, using carbophenothion as the internal standard. It may be necessary to use a second internal standard to identify possible interference with the peak corresponding to carbophenothion.

Table - 3

Substance	*Relative retention times*
Dichlorvos	0.20
Fonofos	0.50
Diazinon	0.52
Parathion-methyl	0.59
Chlorpyrifos-methyl	0.60
Pirimiphos-methyl	0.66
Malathion	0.67
Parathion	0.69
Chlorpyrifos	0.70
Methidathion	0.78
Ethion	0.96
Carbophenothion	1.00
Azinphos-methyl	1.17
Phosalon	1.18

Test solution. Concentrate solution C in a current of *helium for chromatography R* or *oxygen-free nitrogen R* almost to dryness and dilute to 500 µl with *toluene R.*

Reference solution. Prepare at least three solutions in *toluene R* containing the insecticides to be determined and carbophenothion at concentrations suitable for plotting a calibration curve.

The chromatographic procedure may be carried out using:

- a fused silica column 30 m long and 0.32 mm in internal diameter the internal wall of which is covered with a layer 0.25 µm thick of *poly (dimethyl) (diphenyl) siloxane R.*
- *hydrogen for chromatography R* as the carrier gas. Other gases such as *helium for chromatography R* or *nitrogen for chromatography R* may also be used, provided the chromatography is suitably validated.
- an electron-capture detector.
- a device allowing direct cold on-column injection.

maintaining the temperature of the column at 80ºC for 1 min, then raising it at a rate of 30ºC/min to 150ºC, maintaining at 150ºC for 3 min, then raising the temperature at a rate of 4ºC/min to 280ºC and maintaining at this temperature for 1 min and maintaining the temperature of the injector port at 250ºC and that of the detector at 275ºC. Inject the chosen volume of each solution. When the chromatograms are recorded in the prescribed conditions, the relative retention times are approximately those listed in Table-4. Calculate the content of each insecticide from the peak areas and the concentrations of the solutions.

2.6.1. GAS CHROMATOGRAPHY

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives which are volatilized under the temperatures employed.

GC is based on mechanisms of adsorption, mass distribution or size exclusion.

APPARATUS

The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and a data acquisition system (or an integrator or a chart recorder). The carrier gas flows through the column at a controlled rate or pressure and then through the detector.

The chromatography is carried out either at a constant temperature or according to a given temperature programme.

INJECTORS

Direct injections of solutions are the usual mode of injection, unless otherwise

Table-4

Substance	*Relative retention times*
α-Hexachlorocyclohexane	0.44
Hexachlorobenzene	0.45
β-Hexachlorocyclohexane	0.49
Lindane	0.49
δ-Hexachlorocyclohexane	0.54
ε-Hexachlorocyclohexane	0.56
Heptachlor	0.61
Aldrin	0.68
cis-Heptachlor-epoxide	0.76
o,p-'DDE	0.81
α-Endosulfan	0.82
Dieldrin	0.87
p,p-'DDE	0.87
o,p-'DDD	0.89
Endrin	0.91
β-Endosulfan	0.92
o,p-'DDT	0.95
Carbophenothion	1.00
p,p-'DDT	1.02
cis-Permethrin	1.29
trans-Permethrin	1.31
Cypermethrin*	1.40
Fenvalerate*	1.47 and 1.49
Deltamethrin	1.54

*The substance shows several peaks.

prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporization chamber which may be equipped with a stream splitter.

Injections of vapour phase may be effected by static or dynamic head-space injection systems.

Dynamic head-space (purge and trap) injection systems include a sparging device by which volatile substances in solution are swept into an absorbent column maintained at a low temperature. Retained substances are then desorbed into the mobile phase by rapid heating of the absorbent column.

Static head-space injection systems include a thermostatically controlled sample heating chamber in which closed vials containing solid or liquid samples are placed for a fixed period of time to allow the volatile components of the sample to reach equilibrium between the non-gaseous phase and the vapour phase. After equilibrium has been established, a predetermined amount of the head-space of the vial is flushed into the gas chromatograph.

STATIONARY PHASES

Stationary phases are contained in columns which may be:

- a capillary column of fused-silica chose wall is coated with the stationary phase.
- a column packed with inert particles impregnated with the stationary phase.
- a column packed with solid stationary phase.

Capillary columns are 0.1 mm to 0.53 mm in internal diameter (Ô) and 5 m to 60 m in length. The liquid or stationary phase, which may be chemically bonded to the inner surface, is a film 0.1 µm to 5.0 µm thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter (ϕ) of 2 mm to 4 mm. Stationary phases usually consist of porous polymers or solid supports impregnated with liquid phase.

Supports for analysis of polar compounds on columns packed with low-capacity, low-polarity stationary phase must be inert to avoid peak tailing, The reactivity of support materials can be reduced by silanising prior to coating with liquid phase. Acid-washed, flux-calcinated diatomaceous earth is often used, Materials are available in various particle sizes, the most commonly used particles are in the ranges of 150 µm to 180 µm and 125 µm to 150 µm.

MOBILE PHASES

Retention time and peak efficiency depend on the carrier gas flow rate; retention time is directly proportional to column length and resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in milliliters per minute at atmospheric pressure and room temperature, Flow rate is measured at the detector outlet, either with a calibrated mechanical device or with a bubble tube, while the column is at operating temperature. The linear velocity of the carrier gas through a packed column is inversely proportional to the square root of the internal diameter of the column for a given flow volume. Flow rates of 60 ml/min in a 4 mm internal diameter column and 15 ml/min in a 2 mm internal diameter column, give identical linear velocities and thus similar retention times.

Helium or nitrogen is usually employed as the carrier gas for packed columns,

whereas commonly used carrier gases for capillary columns are nitrogen, helium and hydrogen.

DETECTORS

Flame-ionisation detectors are usually employed but additional detectors which may be used include: electron-capture, nitrogen-phosphorus, mass spectrometric, thermal conductivity, Fourier transform infrared spectrophotometric and others, depending on the purpose of the analysis.

METHOD

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution (s) and the reference solutions (s) as prescribed. The solutions must be free from solid particles.

Criteria for assessing the suitability of the system are described in the chapter on *Chromatographic separation techniques.* The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

Static head-space gas chromatography

Static head-space gas chromatography is a technique particularly suitable for separating and determining volatile compounds present in solid or liquid samples. The method is based on the analysis of the vapour phase in equilibrium with the solid or liquid phase.

APPARATUS

The apparatus consists of a gas chromatograph provided with a device for introducing the sample that may be connected to a module that automatically controls the pressure and the temperature. If necessary, a device for eliminating solvents can be added.

The sample to be analysed is introduced into a container fitted with a suitable stopper and a valve-system, which permits the passage of the carrier gas. The container is placed in a thermostatically controlled chamber at a temperature set according to the substance to be examined.

The sample is held at this temperature long enough to allow equilibrium to be established between the solid or liquid phase and the vapour phase.

The carrier gas is introduced into the container and, after the prescribed time, a suitable valve is opened so that the gas expands towards the chromatographic column taking the volatilized compounds with it.

Instead of using a chromatograph specifically equipped for the introduction of samples, it is also possible to use airtight syringes and a conventional chromatograph. Equilibration is then carried out in a separate chamber and the vapour phase is carried onto the column, taking the precautions necessary to avoid any changes in the equilibrium.

METHOD

Using the reference preparations, determine suitable instrument settings to produce an adequate response.

DIRECT CALIBRATION

Separately introduce into identical containers the preparation to be examined and each of the reference preparations, as prescribed in the monograph, avoiding contact between the sampling device and the samples.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

STANDARD ADDITIONS

Add to a set of identical suitable containers equal volumes of the preparation to be examined. Add to all but one of the containers, suitable quantities of a reference preparation containing a known concentration of the substance to be determined so as to produce a series of preparations containing steadily increasing concentrations of the substance.

Close the containers hermetically and place in the thermostatically controlled chamber set to the temperature and pressure prescribed in the monograph; after equilibration, carry out the chromatography under the prescribed conditions.

Calculate the linear equation of the graph using a least-squares fit and derive from it the concentration of the substance to be determined in the preparation to be examined.

Alternatively, plot on a graph the mean of readings against the added quantity of the substance to be determined. Extrapolate the line joining the points on the graph until it meets the concentration axis. The distance between this point and the intersection of the axes represents the concentration of the substance to be determined in the preparation to be examined.

SUCCESSIVE WITHDRAWALS (MULTIPLE HEAD-SPACE EXTRACTION)

If prescribed, the successive withdrawal method is fully described in the monograph.

2.7.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography (HPLC) is a method of chromatographic separation in which the mobile phase is pumped into a column containing stationary phase by a high-pressure pump system. The test solution injected is carried into the column by the mobile phase. All the components are separated in the column and pass through the detector sequentially. The recorder, integrator or data acquisition system thus records the chromatographic signals.

1. General requirement for the instrument

The HPLC instrument is used here, which should be checked periodically and meets the requirement of relative specification.

(1) *Chromatographic column* The most widely used packing material is the chemically bonded silica gel. Reverse phase chromatographic system uses nonpolar packing materials, in which the most frequently used is octadecylsilane bonded silica gel. Octylsilane and other type of chemically bonded silica gel (such as cyano and amino group bonded silica gel) are also used. Positive-phase chromatographic system uses polar packing material, the most widely used of which is silica gel. The ion-exchange resin is used in ion-exchange chromatography. The gel and macromolecular porous microsphere are used in size exclusion chromatography. Chiral bonded packing materials are used in separation of enantiomers (chiral chromatography).

The characteristics of the packing materials (the shape of the support, the particle size, the pore diameter, the surface area, the surface coverage of the bonded functional group, the carbon content and the bonding mode, etc.) and the pack of the chromatographic column effect directly the retention behavior and separation performance. The packing material with pore diameter less than 15 nm (1 nm=10 Å) is suitable for analysis of the compound whose molecular weight is less than 2000, pore diameter more than 30 nm is suitable for analysis of compound whose molecular weight is more than 2000.

The mobile phase in which the pH value is 2-8 is suitable for the stationary phase with silica gel as the support. If the pH value is more than 8, the silica gel may be dissolved and if the pH value is less than 2, the phase chemically bonded with the silica gel may be broken off. In the case when the mobile phase in which the pH value is more than 8 should be used, the packing material, which is alkali-proof, should be chosen, such as bonded silica gel with high purity silicon as support and a high surface coverage, polymer coated packing material, the organic and inorganic hybridized packing materials and the non-silica gel packing materials. In the case when the mobile phase in which the pH value is less than 2 should be used, the packing material, which is acid-proof, may be chosen. Such as the octadecylsilane modified silica gel with diisopropyl or diisobutyl substitution, which has a bulk mass and thus results in a protection through a steric hindrance, or organic and inorganic hybridized packing material, etc.

(2) *Detectors* The most commonly used detector is the ultraviolet (UV) spectrophotometers, Besides, diode array detectors (DAD), fluorescence spectrophotometers, differential refractometers, evaporative light scattering detectors, electrochemical detectors and mass spectrometers may also be used.

The UV spectrophotometers, diode array detectors, fluorescence spectrophotometers and electrochemical detectors are all selective detectors. The response value is related not only to the concentration of the test solution, but also to the structure of the compound. On the contrary, the differential refractometers and evaporative light scattering detectors are general-purpose detectors; they will respond to all compounds. The response value of the evaporative light scattering detectors for compounds with similar structure is almost related to the mass of the test compound. Diode array detector may record the absorption spectrum within the prescribed

wavelength range simultaneously, thus could be used to measure the spectrum and inspect the purity of the chromatographic peaks.

The response values of the UV spectrophotometers, fluorescence spectrophotometers, electrochemical detectors and differential refractometers are linear with the concentration of the test solution within a certain range, while the response value of the evaporative light scattering detectors is not always linear with the concentration of the test solution, if necessary, if should be transferred mathematically before calculation.

Different detectors have different requirements for the mobile phase. For example, if the UV spectrophotometer is used in the experiment, the mobile phase should at least meet the requirement for the solvent described in the UV spectrophotometry. When the light of lower wavelength is applied for detection, the cut-off wavelength of the organic solvent should be considered and it is better to use chromatographic grade organic solvent. The evaporative light scattering detectors and the mass spectrometers generally do not allow using the mobile phase that contains non-volatile salts.

(3) *Mobile phase:-* As the C_{18} chain is not able to keep an spread state in the water solution, the ration of the organic solvent of the mobile phase should not less than 5%, in the reverse phase chromatographic system of which the stationary phase is octadecylsilane bonded silica gel. Otherwise the random curl of the C_{18} chain will lead to the change of the retention value of the component, which may result in the instability of the chromatographic system.

The type of the stationary phase, the composition of the mobile phase and the mode of the detectors specified under the monograph should not be changed. Others, such as the inner diameter, the length, the brand of the stationary phase and the particle size of the support, the flow rate of the mobile phase and the ratio of each component in the mixed mobile phase, the time span in the gradient elution program, the temperature of the column, the injection volume and the sensitivity of the detectors could be changed appropriately to meet the requirement of the system suitability test. But for some certain monographs, in which only the specific brand of packing material is able to satisfy the requirement of the separation, clear indications should be given under them.

2. The system suitability test.

The suitability test of the chromatographic system generally includes four indexes, number of theoretical plates, resolution, repeatability and tailing factors, in which the resolution and the repeatability are moiré practical indexes.

To carry out the suitability test of the chromatographic system according to the requirement under the individual monograph is to test the chromatographic system by using specific reference substance. The separation conditions of the chromatography should be adjusted in case if the requirement cannot be met.

(3) *Repeatability:-* Inject the reference solution described under the monograph for 5 times successively. Unless specified otherwise, the relative standard deviation of the measured value of the peak areas should be no more than 2.0%.

3. Procedure

(5) *Peak area normalization method.*

As the deviation of this method is so large that it can be only used to roughly inspect the contents of impurities in the substance being examined. It is not suitable for the determination of minute impurities, unless specified otherwise. In this method, the peak area of the impurities and the total peak area on the chromatogram except the solvent peak are measured. Calculate the peak area of each impurity and the percentage of their sum in the total peak area.

2.8.1. TEST FOR AFLATOXINS

Caution – Aflatoxins are highly dangerous and extreme care should be exercised in handling aflatoxin materials.

This test is provided to detect the possible presence of aflatoxins B_1, B_2, G_1 and G_2 in any material of plant origin. Unless otherwise specified in the individual monograph, use the following method.

Zinc Acetate – Aluminum Chloride Reagent – Dissolve 20 g of zinc acetate and 5 g of aluminum chloride in sufficient water to make 100 ml.

Sodium Chloride Solution – Dissolve 5 g of sodium chloride in 50 ml of water.

Test Solution 1 – Grind about 200 g of plant material to a fine powder. Transfer about 50 g of the powdered material, accurately weighed, to a glass-stoppered flask. Add 200 ml of a mixture of methanol and water (17 : 3). Shake vigorously by mechanical means for not less than 30 minutes and filter. [Note – If the solution has interfering plant pigments, proceed as directed for *Test Solution 2.]* Discard the first 50 ml of the filtrate and collect the next 40-ml portion. Transfer the filtrate to a separatory funnel. Add 40 ml of *Sodium Chloride Solution* and 25 ml of solvent hexane and shake for 1 minute. Allow the layers to separate and transfer the lower aqueous layer to a second separatory funnel. Extract the aqueous layer in the separatory funnel twice, each time with 25 ml of methylene chloride, by shaking for 1 minute. Allow the layers to separate each time, separate the lower organic layer and collect the combined organic layers in a 125 ml conical flask. Evaporate the organic solvent to dryness on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup Procedure;* otherwise, dissolve the residue obtained above in 0.2 ml of a mixture of chloroform and acetonitrile (9.8 : 0.2) and shake by mechanical means if necessary.

Test Solution 2 – Collect 100 ml of the filtrate from the start of the flow and transfer to a 250 ml beaker. Add 20 ml of *Zinc Acetate-Aluminum Chloride Reagent* and 80 ml of water. Stir and allow to stand for 5 minutes. Add 5 g of a suitable filtering aid, such as diatomaceous earth, mix and filter. Discard the first 50 ml of the filtrate, and collect the next 80 ml portion. Proceed as directed for *Test Solution 1,* beginning with "Transfer the filtrate to a separatory funnel."

Cleanup Procedure – Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10 mm x 300 mm chromatographic tube. Prepare a slurry of 2 g of silica gel with a mixture of ethyl ether and solvent hexane (3 : 1), pour the slurry into the column and wash with 5 ml of the same solvent mixture. Allow the absorbent to settle and add to the top of the column a layer of 1.5 g of anhydrous sodium sulfate. Dissolve the residue obtained above in 3 ml of methylene chloride and transfer it to the column. Rinse the flask twice with 1-ml portions of methylene chloride, transfer the rinses to the column and elute at a rate not greater than 1 ml per minute. Add successively to the column 3 ml of solvent hexane, 3 ml of ethyl ether and 3 ml of methylene chloride; elute at a rate not greater than 3 ml per minute; and discard the eluates. Add to the column 6 ml of a mixture of methylene chloride and acetone (9 : 1) and elute at a rate not greater than 1 ml per minute, preferably without the aid of

vacuum. Collect this eluate in a small vial, add a boiling chip if necessary and evaporate to dryness on a water bath. Dissolve the residue in 0.2 ml of a mixture of chloroform and acetonitrile (9.8 : 0.2) and shake by mechanical means if necessary.

Aflatoxin Solution – [*Caution – Aflatoxins are highly toxic. Handle with care.*] Dissolve accurately weighed quantities of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 in a mixture of chloroform and acetonitrile (9.8 : 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B_1 and aflatoxin G_1 and 0.1 µg per ml each of aflatoxin B_2 and aflatoxin G_2.

Procedure – Separately apply 2.5 µL, 5 µL, 7.5 µL and 10 µL of the Aflatoxin Solution and three 10-µL applications of either *Test Solution 1* or *Test Solution 2* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Superimpose 5 µL of the *Aflatoxin Solution* on one of the three 10-µL applications of the *Test Solution.* Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85 : 10 : 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the platy by examination under UV light at 365 nm: the four applications of the *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots; the spot obtained from the *Test Solution* that was superimposed on the *Aflatoxin Solution* is no more intense than that of the corresponding *Aflatoxin Solution;* and no spot from any of the other *Test Solutions* corresponds to any of the spots obtained from the applications of the *Aflatoxin Solution.* If any spot of aflatoxins is obtained in the *Test Solution,* match the position of each fluorescent spot of the *Test Solution* with those of the *Aflatoxin Solution* to identify the type of aflatoxin present. The intensity of the aflatoxin spot, if present in the *Test Solution,* when compared with that of the corresponding aflatoxin in the *Aflatoxin Solution* will give an approximate concentration of aflatoxin in the *Test Solution.*

APPENDIX –3

3.1. PHYSICAL TESTS AND DETERMINATIONS

3.1.1. Powder Fineness

The degree of coarseness or fineness of a powder is expressed by reference to the nominal mesh aperture size of the sieves for measuring the size of the powders. For practical reasons, the use of sieves, Appendix 1.2, for measuring powder fineness for most pharmaceutical purposes, is convenient but device other than sieves must be employed for the measurement of particles less than 100 mm in nominal size.

The following terms are used in the description of powders :

Coarse powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 µm.

Moderately coarse powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 710 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 250 µm.

Moderately fine powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 355 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperature of 180 µm.

Fine powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 180 µm.

Very fine powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 125 µm.

When the fineness of a powder is described by means of a number, it is intended that all the particles of the powder shall pass through a *sieve* of which the nominal mesh aperture, in µm, is equal to that number.

When a batch of a vegetable drug is being ground and sifted, no portion of the drug shall be rejected but it is permissible except in the case of assays, to withhold the final tailings, if an approximately equal amount of tailings from a preceding batch of the same drug has been added before grinding.

Sieves – Sieves for testing powder fineness comply with the requirements stated under sieves, Appendix 1.2.

Method

(1) **For coarse and moderately coarse powders** – Place 25 to 100 g of the powder being examined upon the appropriate sieve having a close fitting receiving pan and cover. Shake the sieve in a rotary horizontal direction and vertically by tapping on a hard surface for not less than twenty minutes or until sifting is practically complete. Weigh accurately the amount remaining on the sieve and in the receiving pan.

(2) **For fine and very fine powder –** Proceed as described under coarse and moderately coarse powders, except that the test sample should not exceed 25 g and except that the sieve is to be shaken for not less than thirty minutes, or until sifting is practically complete.

Note – Avoid prolonged shaking that would result in increasing the fineness of the powder during the testing.

With oily or other powders, which tend to clog the openings, carefully brush the screen at interval during siftings. Break up any lumps that may form. A mechanical sieve shaker, which reproduces the circular and tapping motion given to sieves in hand sifting but has a uniform mechanical action may be employed.

3.1.2. Refractive Index

The refractive index (n) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Unless otherwise prescribed, the refractive index is measured at 25º(±0.5) with reference to the wavelength of the D line of sodium (=589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

The Abbe refractometer is convenient for most measurements of refractive index but other refractometer of equal or greater accuracy may be used. Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light.

To achieve accuracy, the apparatus should be calibrated against *distilled water :* which has a refractive index of 1.3325 at 25º or against the reference liquids given in the following table:-

Table

Reference Liquid	$n_D^{20°}$	*Temperature Co-efficient*
		$\Delta n/\Delta t$
Carbon tetrachloride	1.4603	-0.00057
Toluene	1.4969	-0.00056
a-Methylnaphthalene	1.6176	-0.00048

* Reference index value for the D line of sodium, measured at 20º

The cleanliness of the instrument should be checked frequently by determining the refractive index of distilled water, which at 25º is 1.3325.

3.1.3. Weight Per Millilitre and Specific Gravity

Weight per millilitre – The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25º, unless otherwise specified.

Method

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled *Water* at 25º and weighing the contents. Assuming that the weight of 1 ml of *water* at 25º when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 20º and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25º, remove any excess of the substance and weigh. Substract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

Specific gravity – The specific gravity of a liquid is the weight of a given volume of the liquid at 25º (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighings being taken in air.

Method

Proceed as described under Wt. Per ml. Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determined at 25º unless otherwise directed in the individual monograph.

3.1.4. Determination of pH values

The pH value of an aqueous liquid may be defined as the common logarithum of the reciprocal of the hydrogen ion concentration expressed in g, per litre. Although this definition provides a useful practical means for the quantitative indication of the acidity or alkalinity of a solution, it is less satisfactory from a strictly theoretical point of view. No definition of pH as a measurable quantity can have a simple meaning, which is also fundamental and exact.

The pH value of a liquid is determined potentiometrically by means of the glass, electrode and a suitable pH meter.

The reagents used in the determinations are described below:

Method

Operate the pH meter and electrode system according to the manufacturer's instructions. Standardise the meter and electrodes with 0.05 M potassium hydrogen phthalate (pH 4.00) when measuring an acid solution, or with 0.05 M sodium borate when measuring an alkaline solution. At the end of a set of measurement, take a reading of the solution used to standardize the meter and electrodes. This reading

should not differ by more than 0.02 from the original value at which the apparatus was standardized. If the difference is greater than 0.05, the set of measurements must be repeated. The pH/ e.m.f. relationship of the particular glass electrode in use must be checked. The pH/follows; standardize with 0.05 M sodium borate. When the reading is higher by 0.02 or more, or over by 0.05 or more than the appropriate value in the Table, correct the pH values of all solutions measured on that day, assuming the e.m.f. of the glass electrode cell to be linearly related to the pH value of the solution which it contains. Unless otherwise stated all solution must be brought to laboratory temperature prior to measurement. Whilst the pH/temperature coefficient of 0.05 M potassium hydrogen phthalate may be neglected that of 0.05 M sodium borate must be taken into account in accordance with the value given in the Table. When measuring pH values above 10.0 make sure that the glass electrode is suitable for use at the alkaline and of the pH scale and apply any correction that is necessary.

Table
pH value of 0.5 M sodium borate

Temperature	10º	15º	20º	25º	40º
pH	9.29	9.26	9.22	9.18	9.14

Water used as the solvent in the determination of the pH of a solution is water having a pH of 5.5 to 7.0.

Solution of Standard pH

Solutions from pH 1.2 to pH 2.2 are prepared by mixing 50 ml of 0.2 M potassium chloride with quantities of 0.2 N hydrochloric acid, specified in the following table, and diluting with freshly boiled and cooled water to produce 200 ml:-

pH	*ml of 0.2 N hydrochloric acid*
1.2	64.5
1.3	41.5
1.6	26.3
1.7	16.6
2.0	10.6
2.2	06.7

solution from pH 2.2 to pH 3.8 are prepared by mixing 50 ml of 0.2 M potassium hydrogen phithalate with the quantities of 0.2 N hydrochloric acid, specified in the following table, and diluting with freshly boiled and cooled water to produce 200 ml:-

pH	*ml of 0.2 N hydrochloric acid*
2.2	46.70
2.3	39.60
2.6	32.95
2.7	26.42
3.0	20.32
3.2	14.70
3.3	9.90
3.6	5.97
3.7	2.62

Solutions from pH 4.0 to pH 6.2 are prepared by mixing 50 ml of 0.2 M potassium hydrogen phthalate with the quantities of 0.2 N sodium hydroxide, specified in the following table, and diluting with freshly boiled and cooled water to produce 200 ml:-

pH	*ml of 0.2 N sodium hydroxide*
4.0	0.40
4.2	3.70
4.4	7.50
4.6	12.15
4.7	17.70
4.9	20.75
5.0	23.85
5.1	26.95
5.2	29.95
5.3	35.45
5.4	26.45
5.6	39.95
5.8	43.00
6.0	45.45
6.2	47.00

Solutions from pH 5.8 to pH 8.0 are prepared by mixing 50 ml of 02. M potassium hydrogen phosphate with the quantities of 0.2 N sodium hydroxide specified in the following table, and diluting with freshly boiled and cooled water to produce 200 ml:-

pH	*Ml of 0.2 N sodium hydroxide*
5.8	3.72
6.0	5.70
6.2	8.60
6.4	12.60
6.6	17.80
6.8	23.65
7.0	29.63
7.2	35.00
7.4	39.50
7.6	42.80
7.8	45.20
8.0	46.80

Solutions from pH 7.8 of pH 10.0 are prepared by mixing 50 ml of 0.2 M boric acid-potassium chloride with the quantities of 0.2 N sodium hydroxide, specified in the following table, and diluting with freshly boiled and cooled water to produce 20 ml:-

pH	*ml of 0.2 N sodium hydroxide*
7.8	2.61
8.0	3.97
8.2	5.90
8.4	8.50
8.6	12.00
8.8	16.30
9.0	21.30
9.2	26.70
9.4	32.00
9.6	36.85
9.8	30.80
10.0	43.90

Solutions of Standard pH must be kept in glass stopped bottles of alkali-free glass preferably coated with paraffin internally.

3.2. DETERMINATION OF MELTING RANGE AND BOILING RANGE

3.2.1. Determination of Melting Range

The melting-range of a substance is the range between the corrected temperature at which the substance begins to form droplets and the corrected temperature at which it completely melts, as shown by formation of a meniscus.

Apparatus:

(a) A capillary tube of soft glass, closed at one end, and having the following dimensions:

(i) thickness of the wall, about 0.10 to 0.15 mm.

(ii) length about 10 cm or any length suitable for apparatus used.

(iii) internal diameter 0.9 to 1.1 mm for substances melting below 100º or 0.8 to 1.2 mm for substances melting above 100º.

Thermometers:

Accurately standardized thermometers covering the range 10º to 300º, the length of two degrees on the scale being not less than 0.8 mm. These thermometers are of the mercury-in-glass, solid-stem type; the bulb is cylindrical in shape, and made of approved thermometric glass suitable for the range of temperature covered; each thermometer is fitted with a safety chamber. The smallest division on the thermometer scale should vary between 0.1º to 1.5º according to the melting point of the substance under test.

The following form of heating apparatus is recommended.

A glass heating vessel of suitable, construction and capacity fitted with suitable stiring device, capable of rapidly mixing the liquids.

Suitable liquids for use in the heating vessel:

Glycerin	*Upto 150º*
Sulphuric acid to which a small crystal of potassium nitrate or 4 Drops of nitric acid per 100 ml has been added	Upto 200º
A liquid paraffin of sufficiently high boiling range	Upto 250º
Seasame oil	Upto 300º
30 parts of potassium sulphate, dissolved by heating in 70 parts of sulphuric acid	Upto 300º

Any other apparatus or method, preferably, the electric method may be used subject to a check by means of pure substances having melting temperature covering the ranges from 0º to 300º and with suitable intervals.

The following substances are suitable for this purpose.

Substance	*Melting range*
Vanillin	81º to 83º
Acetanilide	114º to 116º
Phenacetin	134º to 136º
Sulphanilamide	164º to 166.5º
Sulphapyridine	191º to 193º
Caffeine (Dried at 100º)	234º to 237º

PROCEDURE

Method I — Transfer a suitable quantity of the powdered and thoroughly dried substance to a dry capillary tube and pack the powder by tapping the tube on a hard surface so as to form a tightly packed column of 2 to 4 mm in height. Attach the capillary tube and its contents to a standardized thermometer so that the closed end is at the level of the middle of the bulb; heat in a suitable apparatus (preferably a round-bottom flask) fitted with an auxiliary thermometer regulating the rise of temperature in the beginning to 3º per minute. When the temperature reached is below the lowest figure of the range for the substance under examination, the heating of the apparatus is adjusted as desired; if no other directions are given, the rate of rise of temperature should be kept at 1º to 2º per minute. The statement 'determined by rapid heating' means that the rate of rise of temperature is 5º per minute during the entire period of heating.

Unless otherwise directed, the temperature at which the substance forms droplets against the side of the tube and the one at which it is completely melted as indicated by the formation of a definite meniscus, are read.

The following emergent stem corrections should be applied to the temperature readings.

Before starting the determination of the melting temperature the auxiliary thermometer is attached so that the bulb touches the standard thermometer at a point midway between the graduation for the expected melting temperature and the surface of the heating material. When the substance has melted, the temperature is read on the auxiliary thermometer. The correction figure to be added to the temperature reading of the standardized thermometer is calculated from the following formula:—

$$0.00015\ N\ (T—t)$$

Where 'T' is the temperature reading of the standardized thermometer.

't' is the temperature reading of the auxiliary thermometer.

'N' is the number of degrees of the scale of the standardized thermometer between the surface of the heating material and level of mercury.

The statement "melting range, a° to b°" means that the corrected temperature at which the material forms droplets must be at least a°, and that the material must be completely melted at the corrected temperature, b°.

Method II—The apparatus employed for this test is the same as described for method I except for such details as are mentioned in the procedure given below:—

Procedure — A capillary tube open at both ends is used for this test. Melt the material under test at as low a temperature as possible. Draw into the capillary a column of the material about 10 mm high. Cool the charged tube in contact with ice for at least 2 hours. Attach the tube to the thermometer by means of rubber band and adjust it in the heating vessel containing water so that the upper edge of the material is 10 mm below the water level. Heat in the manner as prescribed in Method I until the temperature is about 5° below the expected melting point and then regulate the rate of rise of temperature to between 0.5° to 1° per minute. The temperature at which the material is observed to rise in the capillary tube is the melting temperature of the substance.

3.2.2. Determination of Boiling-Range

The billing-range of a substance is the range of temperature within which the whole or a specified portion of the substance distils.

Apparatus:

The boiling-range is determined in a suitable apparatus, the salient features of which are described below:

(a) *Distillation flask* — The flask shall be made of colourless transparent heat-resistant glass and well annealed. It should have a spherical bulb having a capacity of about 130 ml. The side tube slopes downwards in the same plane as the axis of the neck at angle of between 72° to 78°. Other important dimensional details are as under:

Internal diameter of neck	15 to 17 mm
Distance from top of neck to center of side tube	72 to 78 mm
Distance from the center of the side tube to surface of the Liquid when the flask contains 100 ml liquid	87 to 93 mm
Internal diameter of side tube	3.5 to 4.5 mm
Length of side tube	97 to 103 mm

(b) *Thermometer*—Standardised thermometers calibrated for 100 mm immersion and suitable for the purpose and covering the boiling range of the substance under examination shall be employed; the smallest division on the thermometer scale may vary between 0.2° to 1.0° according to requirement.

(c) *Draught Screen*—A suitable draught screen, rectangular in cross section with a hard asbestos board about 6 mm thick closely fitting horizontally to the sides of

the screen, should be used. The asbestos board shall have a centrally cut circular hole, 110 mm in diameter. The asbestos board is meant for ensuring that hot gases from the heat source do not come in contact with the sides or neck of the flask.

(d) *Asbestos Board*—A 150 mm square asbestos board 6 mm thick provided with a circular hole located centrally to hold the bottom of the flask, shall be used. For distillation of liquids boiling below 60° the hole shall be 30 mm in diameter; for other liquid it should be 50 mm in diameter. This board is to be placed on the hard asbestos board of the draught screen covering its 110 mm hole.

(e) *Condenser*—A straight water-cooled glass condenser about 50 cm long shall be used.

Procedure — 100 ml of the liquid to be examined is placed in the distillation flask, and a few glass beads or other suitable substance is added. The bulb of the flask is placed centrally over a circular hole varying from 3 to 5 cm in diameter (according to the boiling range of the substance under examination), in a suitable asbestos board. The thermometer is held concentrically in the neck of the flask by means of a well fitting cork in such a manner that the bulb of the thermometer remains just below the level of the opening of the side-tube. Heat the flask slowly in the beginning and when distillation starts, adjust heating in such a manner that the liquid distils at a constant rate of 4 to 5 ml per minute. The temperature is read when the first drop runs from the condenser, and again when the last quantity of liquid in the flask is evaporated.

The boiling ranges indicated, apply at a barometric pressure of 760 mm of mercury. If the determination is made at some other barometric pressure, the following correction is added to the temperatures read:

$$K - (760 - p)$$

Where p is the barometric pressure (in mm) read on a mercury barometer, without taking into account the temperature of the air;

K is the boiling temperature constant for different liquids having different boiling ranges as indicated below:—

Observed Boiling range	*'K'*
Below 100°	0.04
100° to 140°	0.045
141° to 190°	0.05
191° to 240°	0.055
above 240°	0.06

If the barometric pressure is below 760 mm of mercury the correction is added to the observed boiling-range; if above, the correction is substracted.

The statement 'distils between a° and b°, means that temperature at which the first drop runs from the condenser is not less than a° and that the temperature at which

the liquid is completely evaporated is not greater than b°.

Micro-methods of equal accuracy may be used.

3.3.1. Determination of Optical Rotation and of Specific Optical Rotation.

Optical Rotation: Certain substances, in a pure state, in solution and in tinctures posses the property of rotating the plane of polarized light, i.e., the incident light emerges in a plane forming an angle with the plane of the incident light. These substances are said to be optically active and the property of rotating the plane of polarized light is known as Optical Rotation. The optical rotation is defined as the angle through which the plane of polarized light is rotated when polarized light obtained from sodium or mercury vapour lamp passes through one decimeter thick layer of a liquid or a solution of a substance at a temperature of 25° unless as otherwise stated in the monograph. Substances are described as dextrorotatory or laevoretatory according to the clockwise or anticlockwise rotation respectively of the plane of polarized light. Dextrorotation is designated by a plus (+) sign and laevorotation by a minus (—) sign before the number indicating the degrees of rotation.

Apparatus: A polarimeter on which angular rotation accurate 0.05° can be read may be used.

Procedure: For liquid substances, take a minimum of five readings of the rotation of the liquid and also for an empty tube at the specified temperature. For a solid dissolve in a suitable solvent and take five readings of the rotation of the solution and the solvent used. Calculate the average of each set of five readings and find out the corrected optical rotation from the observed rotation and the reading with the blank (average).

Specific Rotation: The apparatus and the procedure for this determination are the same as those specified for optical rotation.

Specific rotation is denoted by the expression

$$[\alpha] \quad \frac{t}{x}$$

t denotes the temperature of rotation; α denotes the wave length of light used or the characteristic spectral line. Specific rotations are expressed in terms of sodium light of wave length 589.3 mw (D line) and at a temperature of 25°, unless otherwise specified.

Specific rotation of a substance may be calculated from the following formulae: For liquid substances

$$[\alpha]^t = \frac{a}{ld}$$

For solutions of substances

$$[\alpha]^t \leftrightarrow = \frac{a \times 100}{lc}$$

Where a is the corrected observed rotation in degrees 1 is the length of the polarimeter tube in decimeters.

D is the specific gravity of the liquid C is the concentration of solution expressed as the number of g of the substance in 100 ml of solution.

Specific Rotation : 1. Apomorphinum muriaticum
Determined in a solution containing the equivalent of 0.15 g of anhyd. Apomorphine in 10 ml of 0.02N HCl. The optical rotation should be not less than -49^{o} and not more than -52^{o}.

2. Chinium muriaticum
Dissolve 0.5 g in 0.1 N hydrochloric acid and dilute to 25 ml with the same solvent. The optical rotation should be not less than—240^{o} and not more than -258^{o}.

3. Codeinum
Dissolve 0.5 g in alcohol and dilute to 25 ml with the same solvent. The optical rotation should be in between -142^{o} and -146^{o}.

Optical Rotation : Copaiba Officinalis—Essential oil—optical rotation should be in between -7^{o} and -35^{o}.

3.3.2. Disintegration test:

Apparatus — A glass or suitable plastic tube 80—100—mm long, with an internal diameter of about 28 mm and an external diameter of 30 to 31 mm fitted at the lower end with a disc of rust proof wire gauze complying with the requirements for a No. 10 sieve, is suspended in a volume of *water*, having a depth not less than 15cm and at a temperature between 35^{o} and 39^{o}, in such a way that it can be raised and lowered repeatedly in a uniform manner through a distance of 75 mm; at the highest position of the tube, the gauze just breaks the surface of the *water* and at the lowest position the upper rim of the remains clear of the *water*. The tube may be manipulated by hand or mechanically.

Guided disc — This consists of a disc of a suitable plastic material, about 26 mm in diameter and 2 mm thick; the lower surface is flat and the upper surface has three holes equally spaced and 10mm from the center. In each hole stainless steel wire of no. 22 standard wire gauze is secured at a right angle to the plane of the disc and at the end of each wire is turned out radically and secured to a guide ring 27 mm in diameter, made of similar material. The guide ring is coaxial with the disc from a parallel plane at distance of 15 mm from the upper surface of the disc. The difference between diameter of the disc and the internal diameter of the tube is not more than 2 mm. The total weight of the guided disc is not less than 1.0 g and not more than 2.1 g.

Method — Place five tablets in the tube. Insert the guided disc above the tablets, in the tube and raise and lower the tube in such a manner that the complete up and

down movement is repeated thirty times a minute. The tablets are disintegrated when no particles remains above the gauze which will not readily pass through it. The time required for five tablets to disintegrate in the manner prescribed is, unless otherwise stated in the monograph, not more than fifteen minutes.

Whenever the medicated tablets are concerned they should in addition to stated quantity of drug respond favourably to the standard prescribed for tablets.

Disintegration: This test is not applicable to modified-release tablets for use in the mouth. For those tablets for which the *dissolution test for tablets and capsules,* is included in the individual monograph, the test for Disintegration is not required.

Uncoated Vattis /Tablets: Comply with the *disintegration test for tablets and capsules,* Unless otherwise directed in the individual monograph, use *water* as the medium and add a disc to each tube. Operate the apparatus for 15 minutes unless otherwise directed.

Coated Vattis /Tablets: Comply with the *disintegration test for tablets and capsules,* Unless otherwise directed in the individual monograph, use *water* as the medium and add a disc to each tube. Operate the apparatus for 30 minutes for film-coated tablets and for 60 minutes for guggulu or other coated tablets unless otherwise directed in the individual monograph. For coated tablets other than film-coated tablets, if any of the tablets have not disintegrated, repeat the test on a further 6 tablets, replacing the *water* in the vessel with *0.1 M hydrochloric acid.* The tablets comply with the test if all 6 tablets have disintegrated in the acid medium.

Enteric-coated Vattis /Tablets: Comply with the *disintegration test for tablets and capsules.* If the tablet has a soluble external coating, immerse the basket in *water* at room temperature for 5 minutes. Suspend the assembly in the beaker containing *0.1 M hydrochloric acid* and operate without the discs for 60 minutes, unless otherwise stated in the individual monograph. Remove the assembly from the liquid. No tablet shows signs of cracks that would allow the escape of the contents of disintegration, apart from fragments of coating. Replace the liquid in the beaker with *mixed phosphate buffer pH 6.8,* add a disc to each tube and operate the apparatus for a further 60 minutes. Remove the assembly from the liquid. The tablets pass the test if all six have disintegrated.

Dispersible and Soluble Vattis/ Tablets: Disintegrate within 3 minutes when examined by the *disintegration test for tablets and capsule,* using *water* at 24º to 26º, unless otherwise stated in the individual monograph.

Effervescent Vattis /Tablets: Place one tablet in a 250-ml beaker containing *water* at 20º to 30º; numerous gas bubbles are evolved. When the evolution of gas around the tablet or its fragments has ceased the tablet shall have disintegrated, being either dissolved or dispersed in the water so that no agglomerates of particles remain. Repeat the operation on a further 5 tablets. The tablets comply with the test if each of the 6 tablets disintegrates in the manner prescribed within 5 minutes, unless otherwise stated in the individual monograph.

Uniformity of dispersion: This test is applicable only to Dispersible Tablets. Place 2 tablets in 100 ml of *water* and stir gently until completely dispersed. A smooth

dispersion is obtained which passes through a sieve screen with a nominal mesh aperture of 710 µm (*sieve number 22).*

3.4.1. UNIFORMITY OF WEIGHT

This test is applicable to tablets that contain less than 10 mg or less than 10% w/w of active ingredient. For tablets containing more than one active ingredient carry out the test for each active ingredient that corresponds to the aforementioned conditions.

The test for **Uniformity of content** should be carried out only after the content of active ingredient (s) in pooled sample of the tablets has been shown to be within accepted limits of the stated content.

The test for **Uniformity of content** *is not applicable to tablets containing trace elements.*

Determine the content of active ingredients (s) in each of 10 tablets taken at random using the method given in the monograph or by any other suitable analytical method. The tablets comply with the test if not more than one of the individual values thus obtained is outside the limits 85 to 115% of the average value and none is outside the limits 75 to 125% of the average value. If two or three of the individual values are outside the limits 85 to 115% of the average value and none is outside the limits 75 to 125%, repeat the determination using another 20 tablets. The tablets comply with the test if in the total sample of 30 tablets not more than three of the individual values are outside the limits 85 to 115% and none is outside the limits 75 to 125% of the average value.

3.4.2. DETERMINATION OF VISCOSITY

Viscosity is a property of a liquid, which is closely related to the resistance to flow.

In C.G.S. system, the dynamic viscosity (n) of a liquid is the tangential force in dryness per square centimeter exerted in either of the two parallel planes placed, 1 cm apart when the space between them is filled with the fluid and one of the plane is moving in its own plane with a velocity of 1 cm per second relatively to the other. The unit of dynamic viscosity is the poise (abbreviated p). The centi poise (abbreviated cp) is $1/100^{th}$ of one poise.

While on the absolute scale, viscosity is measured in poise or centi poise, it is mot convenient to use the kinematic scale in which the units are stokes (abbreviated S) and centi-stokes (abbreviated CS). The centistokes is $1/100^{th}$ of one stoke. The kinematic viscosity of a liquid is equal to the quotient of the dynamic viscosity and the density of the liquid at the same temperature, thus :

$$\text{Kinematic Viscosity} = \frac{\text{Dynamic Viscosity}}{\text{Density}}$$

Viscosity of liquid may be determined by any method that will measure the resistance to shear offered by the liquid.

Absolute viscosity can be measured directly if accurate dimensions of the measuring instruments are known but it is more common practice to calibrate the instrument with a liquid of known viscosity and to determine the viscosity of the unknown fluid by comparison with that of the known.

Procedure — The liquid under test is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified weight of the viscometer and the time taken for the meniscus to pass the two specified marks is measured. The kinematic viscosity in centistokes is calculated from the following equation:

Kinematic viscosity = kt

Where k = the constant of the viscometer tube determined by observation on liquids of known kinematic viscosity. T = time in seconds for meniscus to pass through the two specified marks.

3.5.1. TOTAL SOLIDS

Determination of total solids in Asava/ Aristha is generally required. Asava/ Aristha containing sugar or honey should be examined by method 1, sugar or honey free Asava/ Aristha and other material should be examined by method 2.

Method 1 Transfer accurately 50 ml of the clear Asava/ Aristha an evaporable dish and evaporate to a thick extract on a water bath. Unless specified otherwise, extract the residue with 4 quantities, each of 10 ml, of dehydrated ethanol with stirring and filter. Combine the filtrates to another evaporating dish which have been dried to a constant weight and evaporate nearly to dryness on a water bath, add accurately 1 g of diatomite (dry at 105ºC for 3 hours and cooled in a desiccator for 30 min), stir thoroughly, dry at 105ºC for 3 hours, cool the dish in a desiccator for 30 min, and weigh immediately. Deduct the weight of diatomite added, the weight of residue should comply with the requirements stated under the individual monograph.

Method 2 Transfer accurately 50 ml of the clear Asava/ Aristha to an evaporable dish, which have been dried to a constant weight and evaporate to dryness on a water bath, then dry at 105ºC for 3 hours. After cooling the dish containing the residue in a desiccator for 30 min, weigh it immediately. The weight of residue should comply with the requirements stated under the individual monograph.

3.6. DETERMINATION OF SAPONIFICATION, IODINE & ACID VALUES

3.6.1. Determination of Saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat, when determined by the following method :—

Dissolve 35 to 40 g of potassium hydroxide in 20 ml water, and add sufficient alcohol to make 1,000 ml. Allow it to stand overnight, and pour off the clear liquor.

Weigh accurately about 2 g of the substance in a tared 250 ml flask, add 25 ml of the alcoholic solution of potassium hydroxide, attach a reflux condenser and boil on a water-bath for one hour, frequently rotating the contents of the flask cool and add 1 ml of solution of phenolphthalein and titrate the excess of alkali with 0.5 N hydrochloric acid. Note the number of ml required (a) Repeat the experiment with the same quantities of the same reagents in the manner omitting the substance. Note the number of ml required (b) Calculate the saponification value from the following formula:—

$$\text{Saponification Value} = \frac{(b-a) \times 0.02805 \times 1.000}{W}$$

Where 'W' is the weight in g of the substance taken.

3.6.2. Determination of Iodine Value

The Iodine value of a substance is the weight of iodine absorbed by 100 part by weight of the substance, when determined by one of the following methods:-

Apparatus

Iodine Flasks—The Iodine flasks have a nominal capacity of 250 ml.

Method:— Iodine Monochloride Method—Place the substance accurately weighed, in dry iodine flask, add 10 ml of carbon tetrachloride, and dissolve. Add 20 ml of iodine monochloride solution, insert the stopper, previously moistened with solution of potassium iodine and allow to stand in a dark place at a temperature of about 17^{o} or thirty minutes. Add 15 ml of solution of potassium iodine and 100 ml water; shake, and titrate with 0.1 N sodium thiosulphate, using solution of starch as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of ml of 0.1 N sodium thiosulphate required (b).

Calculate the iodine value from the formula:—

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{W}$$

Where 'W' is the weight in g of the substance taken.

The approximate weight, in g, of the substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

Reagent:—

Iodine Monochloride Solution—The solution may be prepared by either of the two following methods:

(1) Dissolve 13 g of iodine in a mixture of 300 ml of carbon tetrachloride and 700 ml of glacial acetic acid. To 20 ml of this solution, add 15 ml of solution of potassium

iodide and 100 ml of water, and titrate the solution with 0.1 N sodium thiosulphate. Pass chlorine, washed and dried, through the remainder of the iodine solution until the amount of 0.1 N sodium thiosulphate required for the titration is approximately, but more than, doubled.

(2)

Iodine Trichloride	8 g
Iodine	9 g
Carbon Tetrachloride	300 ml
Glacial Acetic Acid, sufficient to produce	1000 ml

Dissolve the iodine trichloride in about 200 ml of glacial acetic acid, dissolve the iodine in the carbon tetrachloride, mix the two solutions, and add sufficient glacial acetic acid to produce 1000 ml/ Iodine Monochloride Solution should be kept in a stoppered bottle, protected from light and stored in a cool place.

Pyridine Bromide Method — Place the substance, accurately weighed, in a dry iodine flask, add 10 ml of carbon tetrachloride and dissolve. Add 25 ml of pyridine bromide solution, allow to stand for ten minutes in a dark place and complete the determination described under iodine monochloride method, beginning with the words. Add 15 ml.

The approximate weight in gram, of the substance to be taken may be calculated by dividing 12.5 by the highest expected iodine value. If more than half the available halogen is absorbed the test must be repeated, a small quantity of the substance being used.

Reagent: —

Pyridine Bromide Solution—Dissolve 8 g pyridine and 10 g of sulphuric acid in 20 ml of glacial acetic acid, keeping the mixture cool. Add 8 g of bromine dissolved in 20 ml of glacial acetic acid and dilute to 100 ml with glacial acetic acid.

Pyridine Bromide Solution should be freshly prepared.

3.6.3. Determination of Acid Value—

The acid value is the number of mg potassium hydroxide required to neutralize the free acid in 1 g of the substance, when determined by the following method:—

Weigh accurately about 10 g of the substance (1 to 5) in the case of a resin into a 250 ml flask and add 50 ml of a mixture of equal volumes of alcohol and solvent ether, which has been neutralized after the addition of 1 ml of solution of phenolphthalein. Heat gently on a water-bath, if necessary until the substance has completely melted, titrate with 0.1 N potassium hydroxide, shaking constantly until a pink colour which persists for fifteen seconds is obtained. Note the number of ml required. Calculate the acid value from the following formula: —

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

Where 'a' is the number of ml. of 0.1 N potassium hydroxide required and 'w' is the weight in g of the substance taken.

3.6.4. Determination of Peroxide Value

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

Method

Unless otherwise specified in the individual monograph, weigh 5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of *glacial acetic acid* and 2 volumes of *chloroform,* swirl until dissolved and add 0.5ml volumes of *saturated potassium iodide soluton.* Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of *water* and titrate gradually, with continuous and vigorous shaking, with *0.01M sodium thiosulphate* until the yellow colour almost disappears. Add 0.5 ml of *starch solution* and continue the titration, shaking vigorously until the blue colour just disappears (a ml). Repeat the operation omitting the substance being examined (b ml). The volume of *0.01M sodium thiosulphate* in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression

Peroxide value = $10 (a - b)/w$

Where w = weight, in g, of the substance.

3.6.5. Determination of Unsaponifiable Matter

The unsaponifiable matter consists of substances present in oils and fats, which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the saponified substance being examined.

Method

Unless otherwise specified in the individual monograph, introduce about 5 g of the substance being examined, accurately weighed, into a 250-ml flask fitted with a reflux condenser. Add a solution of 2 g of *potassium hydroxide* in 40 ml of *ethanol* (95%) and heat on a water-bath for 1 hour, shaking frequently. Transfer the contents of the flask to a separating funnel with the aid of 100 ml of hot water and, while the liquid is still warm, shake very carefully with three quantities, each of 100 ml, of *peroxide-free ether.* Combine the ether extracts in a second separating funnel containing 40 ml of water, swirl gently for a few minute, allow to separate and reject the lower layer. Wash the ether extract with two quantities, each of 40 ml, of water and with three quantities, each of 40 ml, of a 3% w/v solution of *potassium hydroxide,* each treatment being followed by a washing with 40 ml of water. Finally, wash the ether layer with successive quantities, each of 40 ml, of water until the aqueous layer is not alkaline to *phenolphthalein solution.* Transfer the ether layer to a weighed flask, washing out the separating funnel with *peroxide-free ether.* Distil off the ether and add to the residue 6 ml of *acetone.* Remove the solvent completely from the flask with the aid of a gentle current of air. Dry at 100° to 105° for 30 minutes. Cool in a desiccator and weigh the residue. Calculate the unsaponifiable matter as % w/w.

Dissolve the residue in 20 ml of *ethanol* (95%), previously neutralised to

phenolphthalein solution and titrate with *0.1M ethanolic potassium hydroxide.* If the volume of *0.1M ethanolic potassium hydroxide* exceeds 0.2 ml, the amount weighed cannot be taken as the unsaponifiable matter and the test must be repeated.

3.6.6 Detection of Mineral Oil (Holde's Test):

Take 22ml of the Alcoholic KOH solution in a conical flask and add 1ml of the sample of oil to be tested. Boil in a water bath using an air or water cooled condenser till the solution becomes clear and no oily drops are found on the sides of the flask. Take out the flask from the water bath, transfer the contents to a wide mouthed warm test tube and carefully add 25ml of boiling distilled water along the side of the test tube. Continue shaking the tube lightly from side to side during the addition. The turbidity indicates presence of mineral oil, the depth of turbidity depends on the percentage of mineral oil present

3.6.7 Rancidity test (Kreis Test)

The test depends upon the formation of a red colour when oxidized fat is treated with conc. HCl and a solution of phloroglucinol in ether. The compound in rancid fats responsible for the colour reaction is epihydrin aldehyde. All oxidized fats respond to the Kreis test and the intensity of the colour produced is roughly proportional to the degree of oxidative rancidity.

Procedure

Mix 1 ml of melted fat and 1 ml of conc. HCl in a test tube. Add 1 ml of a 1 % solution of phloroglucinol in diethyl ether and mix thoroughly with the fat-acid mixture. A pink colour formation indicates that the fat is slightly oxidized while a red colour indicates that the fat is definitely oxidized.

3.6.8 Determination Of Reichert-Meissl And Polenske Value

Definition:

The Reichert-Meissl value is the number of millitres of 0.1N aqueous sodium hydroxide solution required to neutralize steam volatile water soluble fatty acids distilled from 5g of an oil/fat under the prescribed conditions. It is a measure of water soluble steam volatile fatty acids chiefly butric and caprole acids present in oil or fat.

The Polenske value is the number of millitres of 0.1N aqueous alkali solution required to neutralize steam volatile water insoluble fatty acids distilled from 5 g of the oil/fat under the prescribed conditions. It is a measure of the steam volatile and water insoluble fatty acids, chiefly caprylic, capric and lauric acids present in oil and fat.

Principle:

The material is saponified by heating with glycerol sodium hydroxide solution and then split by treatment with dilute sulfuric acid. The volatile acids are immediately steam distilled. The soluble volatile acids in the distillate are filtered out and estimated by titration with standard sodium hydroxide solution.

Analytical importance:

These determinations have been used principally for the analysis of butter and margarines. Butter fat contains mainly butyric acid glycerides. Butyric acid is volatile and soluble in water.

No other fat contains butyric acid glycerides, and therefore, the Reichert-Meissl value of the butter fat is higher than that for any other fat. Coconut oil and palm kernel oil contain appreciable quantities of caprylic, capric and lauric acid glyceride. These fatty acids are steam volatile but not soluble in water, and hence give high Polenske value.

Reagents:

a. Glycerine : Analytical reagent grade

b. Concentrated sodium hydroxide solution: 50 percent (w/w)

c. Pumice stone grains

d. Dilute sulfuric acid solution: Approximately 1.0 N

e. Sodium hydroxide solution: 0.1N solution in water, accurately standardized.

f. Phenolphthalein indicator: Dissolve 0.1g of phenolphthalein in 100 ml of ethyl alcohol

g. Ethyl alcohol : 90% by volume and neutral to phenolphthalein.

Procedure :

Weigh accurately 5 ± 0.1 g of filtered oil or fat sample into a clean, dry, 300 ml distilling flask. Add 20 g of glycerine and 2 ml of concentrated sodium hydroxide solution, and heat with swirling over a flame until completely saponified, as shown by the mixture becoming perfectly clear. Cool the content slightly and add 90 ml of boiling distilled water, which has been vigorously boiled for about 15 min. After thorough mixing the solution should remain clear. If the solution is not clear (indicating incomplete saponification) or is darker than light yellow (indicating over-heating), repeat the saponification with a fresh sample of the oil or fat. If the sample is old , the solution may some times be dark and not clear.

Add about 0.1 g of pumic stone grains, and 50 ml of dilute sulfuric acid solution. Immediately connect the flask to the distillation apparatus. Heat very gently until the liberated fatty acids melt and separate. Then set the flame so that 110 ml of distillate shall be collected within 19 to 21 min. The beginning of the distillation is to be taken as the moment when the first drop forms in the still head. Collect the distallate in a graduated flask. The temperature of the issuing distillate should be between 18° to 21°C.

When the distillate exactly reaches the 110 ml mark on the flask, remove the flame and quickly replace the flask by a 25 ml measuring cylinder. Stopper the graduated flask and without mixing place it in a water bath maintain at 15°C for 10 min so that the 110 ml graduation mark is 1 cm below the water level in the bath. Remove the graduated flask from the cold water bath, dry the outside and mix the content gently

by inverting the flask 4 or 5 times without shaking. Avoid wetting the stopper with the insoluble acids. Filter the liquid through a dry, 9 cm Whatman No.4 filter paper. The filtrate should be clear. Pipette 100 ml of the filtrate and add 5 drops of the phenolophthalein solution, and titrate against standard 0.1 N sodium hydroxide solution. Run a Blank Test without the fat, but using the same quantities of the reagents.

Calculation :

Reichert-Meissl Value = (A-B) x N x 11

where,

A = Volume in ml of standard sodium hydroxide solution required for the test;

B = Volume in ml of standard sodium hydroxide solution required for the blank;

N = Normality of standard sodium hydroxide solution.

After titrating the soluble volatile acids, detach the still head and rinse the condenser with three successive 15 ml portions of cold distilled water passing each washing separately through the measuring cylinder, 110 ml graduated flask and the filter paper and allow all of it to pass through. Discard all the washings. Place the funnel on a clean conical flask. Dissolve the insoluble fatty acids by three similar washings of the condenser, the measuring cylinder, the 110 ml flask with stopper, and the filter paper with 15 ml portions of ethyl alcohol. Combine the alcoholic washings in a clean flask, add 5 drops of phenolphthalein indicator solution, and titrate with standard (0.1N) sodium hydroxide solution.

Polenske Value = 10 x V x N

where

V = Volume in ml of standard sodium hydroxide solution required for the test;

N = Normality of the standard sodium hydroxide solution.

3.7.1. DETERMINATION OF ALCOHOL CONTENT

The ethanol content of a liquid is expressed as the number of volumes of ethanol contained in 100 volumes of the liquid, the volumes being measured at 24.9º to 25.1º. This is known as the "percentage of ethanol by volume". The content may also be expressed in grams of ethanol per 100 g of the liquid. This is known as the 'percentage of ethanol by weight".

Use Method I or Method II, as appropriate, unless otherwise specified in the individual monograph.

Method I

Carry out the method for gas chromatography, Appendix 4.2, using the following solutions. Solution (1) contains 5.0% v/v of ethanol and 5.0% v/v of 1-propanol (internal standard). For solution (2) dilute a volume of the preparation being examined with water to contain between 4.0 and 6.0% v/v of ethanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 5.0% v/v.

The chromatographic procedure may be carried out using a column (1.5 m x 4 mm) packed with porous polymer beads (100 to 120 mesh) and maintained at 150°, with both the inlet port and the detector at 170°, and nitrogen as the carrier gas.

Calculate the percentage content of ethanol from the areas of the peaks due to ethanol in the chromatogram obtained with solutions (1) and (3).

Method II

For preparations where the use of Industrial Methylated Spirit is permitted in the monograph, determine the content of ethanol as described in Method I but using as solution (2) a volume of the preparation being examined diluted with water to contain between 4.0 and 6.0% v/v of total ethanol and methanol.

Determine the concentration of methanol in the following manner. Carry out the chromatographic procedure described under Method I but using the following solutions. Solution (1) contains 0.25% v/v of methanol and 0.25% v/v of 1-propanol (internal standard). For solution (2) dilute a volume of the preparation being examined with water to contain between 0.2% and 0.3% v/v of methanol. Prepare solution (3) in the same manner as solution (2) but adding sufficient of the internal standard to produce a final concentration of 0.25% v/v.

The sum of the contents of ethanol and methanol is within the range specified in the individual monograph and the ration of the content of methanol to that of ethanol is commensurate with Industrial Methylated Spirit having been used.

Method III

This method is intended only for certain liquid preparations containing ethanol. Where the preparation contains dissolved substances that may distil along with ethanol Method IIIB or IIIC must be followed.

Apparatus

The apparatus (see Fig. 1) consists of a round-bottomed flask (A) fitted with a distillation head (B) with a steam trap and attached to a vertical condenser (C). A tube is fitted to the lower part of the condenser and carries the distillate into the lower part of a 100-ml or 250-ml volumetric flask (D). The volumetric flask is immersed in a beaker (E) containing a mixture of ice and water during the distillation. A disc with a circular aperture, 6 cm in diameter, is placed under the distillation flask (A) to reduce the risk of charring of any dissolved substances.

Method IIIA

Transfer 25 ml of the preparation being examined, accurately measured at 24.9° to 25.1°, to the distillation flask. Dilute with 150 ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and collect not less than 90 ml of the distillate into a 100-ml volumetric flask. Adjust the temperature to 24.9° to 25.1° and dilute to volume with distilled water at 24.9° to 25.1°. Determine the relative density at 24.9° to 25.1°, Appendix 8.15. The values indicated in column 2 of Table 1 are multiplied by 4 in order to obtain the percentage of ethanol by volume contained

in the preparation. If the specific gravity is found to be between two values, the percentage of ethanol should be obtained by interpolation. After calculation of the ethanol content, report the result to one decimal place.

Note – (1) If excessive frothing is encountered during distillation, render the solution strongly acid with phosphoric acid or treat with a small amount of liquid paraffin or a silicone oil.

(2) The distillate should be clear or not more than slightly cloudy. If it is turbid or contains oily drops, follow Method IIIC. When steam-volatile acids are present, make the solution just alkaline with 1M sodium hydroxide using solid phenolphthalein as indicator before distillation.

Method IIIB

Follow this method or the following one if the preparation being examined contains appreciable proportions of volatile materials other than ethanol and water.

Mix 25 ml of the preparation, accurately measured at 24.9° to 25.1°, with about 100 ml of water in a separating funnel. Saturate this mixture with sodium chloride, add

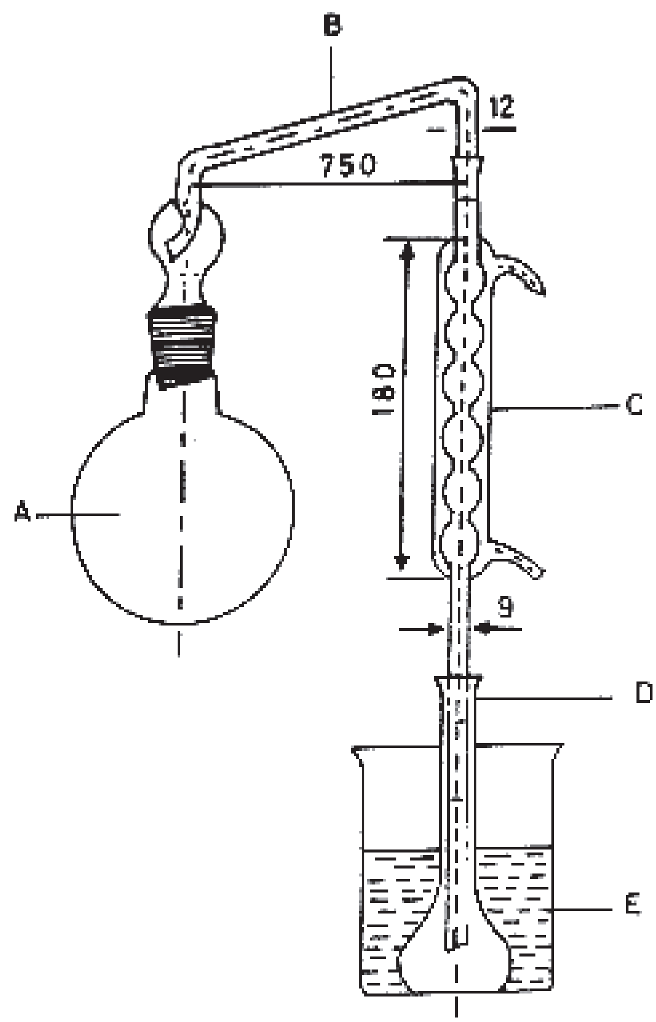


Fig. Apparatus for Determination of Ethanol by Distillation Method

Table 1

Specific gravity at 25º	*Ethanol* content*
1.0000	0
0.9985	1
0.9970	2
0.9956	3
0.9941	4
0.9927	5
0.9914	6
0.9901	7
0.9888	8
0.9875	9
0.9862	10
0.9850	11
0.9838	12
0.9826	13
0.9814	14
0.9802	15
0.9790	16
0.9778	17
0.9767	18
0.9756	19
0.9744	20
0.9733	21
0.9721	22
0.9710	23
0.9698	24
0.9685	25

* % v/v at 15.56º.

about 100 ml of hexane and shake vigorously for 2 to 3 minutes. Allow the mixture to stand for 15 to 20 minutes. Run the lower layer into the distillation flask, wash the hexane layer in the separating funnel by shaking vigorously with about 25 ml of sodium chloride solution, allow to separate and run the wash liquor into the first saline solution.

Make the mixed solutions just alkaline with 1M sodium hydroxide using solid phenolphthalein as indicator, add a little pumice powder and 100 ml of water, distil 90 ml and determine the percentage v/v of ethanol by Method IIIA beginning at the words "Adjust the temperature...".

Method IIIC

Transfer 25 ml of the preparation, accurately measured at 24.9º to 25.1º, to the distillation flask. Dilute with 150 ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and collect about 100 ml. Transfer to a separating funnel and determine the percentage v/v of ethanol by Method IIIB beginning at the words "Saturate this mixture...".

3.8.1. TESTS FOR STERILITY

The tests for sterility are intended for detecting the presence of viable forms of micro-organisms in or on pharmacopoeial preparations. The tests must be carried out under conditions designed to avoid accidental contamination of the product during the test. Precautions taken for this purpose should not adversely affect any micro-organisms which should be revealed in the test.

The working conditions in which the tests are performed should be monitored regularly by sampling the air and surfaces of the working area and by carrying out control tests, The tests are based upon the principle that if micro-organisms are placed in a medium which provides nutritive material and water, and kept at a favourable temperature, the organisms will grow and their presence can be indicated by a turbidity in the originally clear medium.

The probability of detecting viable micro-organisms in the *test for sterility* increases with the number present in a given amount of the preparation being examined and varies according to the species of micro-organisms present. Very low levels of contamination cannot be detected on the basis of random sampling of a batch. (A batch may be defined for the purposes of these tests as a homogeneous collection of) sealed containers prepared in such a manner that the risk of contamination is the same for each of the units in it). Moreover, if contamination is not uniform throughout the batch, random sampling cannot detect contamination with certainty. Compliance with the *tests*

Table 1

Number of items in the batch	*Minimum number of items recommended to be tested*
1. *Ophthalmic and other non-injectable preparations*	
Not more than 200 containers	5% or 2 containers whichever is greater
More than 200 containers	10 containers
2. *Surgical dressings*	10% or 4 packages whichever
Not more than 100 packages	is greater
More than 100 but not more than 500 packages	10 packages
More than 500 packages	2% or 20 packages whichever is less
3. *Bulk solids*	
Less than 4 containers	Each container
4 containers but not more than 50 containers	20% or 4 containers whichever is greater
More than 50 containers	2% or 10 containers whichever is greater

for sterility alone cannot therefore constitute absolute assurance of freedom from microbial contamination. Greater assurance of sterility must come from reliable manufacturing procedures and compliance with Good Manufacturing Practices.

The *tests for sterility* are designed to reveal the presence of micro-organisms in the samples used in the tests; interpretation of results is based on the assumption that the contents of every container in the batch, had they been tested, would also have complied with the the tests. Since every container cannot be tested, a sufficient number of containers should be examined to give a suitable degree of confidence in the results of the tests.

No sampling plan for applying the tests to a specified proportion of discrete units selected from a batch is capable of demonstrating that all of the untestred units are in fact sterile. Therefore, in determining the number of units to be tested, the manufacturer should have regard to the environmental conditions of manufacture, the volume of preparation per container and other special considerations particular to the preparation being examined. Table 1 gives guidance on the minimum number of items recommended to be tested in relation to the number of items in the batch on the assumption that the preparation has been manufactured under conditions designed to exclude contamination.

3.8.2. Culture Media

Media for the tests may be prepared as described below, or dehydrated mixtures yielding similar formulations may be used provided that when reconstituted as directed by the manufacturer, they have growth-promoting properties equal to or superior to those from the formulae given herein. Other media may be used provided that they have been shown to sustain the growth of a wide range of micro-organisms.

Fluid thioglycollate medium – For use with clear fluid products.

$_L$-Cystine	0.5 g
Sodium chloride	2.5 g
Dextrose ($C_6H_{12}O_6,H_2O$)	5.5 g
Granular agar (moisture less than 15% w/w)	0.75 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycollic acid	0.3 ml
Resazurin (0.10% fresh solution)	1.0 ml
Distilled water to	1000 ml

Mix the ingredients other than the thioglycollate and the resazurin, in the order given above in a mortar, with thorough grinding. Stir in some heated distilled water, transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water-bath. Add the sodium thioglycollate, then 1M sodium hydroxide, if necessary, so that (after sterilization) the medium will have a pH

of 7.1±0.2. Reheat the solution, but do not boil, filter (if necessary) through a moistened filter paper and add the resazurin solution.

Distribute into suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a colour change, indicative of oxygen uptake at the end of the incubating period. Sterilise in an autoclave at 121° for 20 minutes. Cool promptly to 25° and store at 20° to 30°, avoiding excess of light. If more than the upper one third has acquired a pink colour, the medium may be restored once by reheating in a water-bath until the pink colour disappears and cooling rapidly. When ready for use, not more than the upper one-tenth of the medium should have a pink colour. Medium more than 3 weeks old should not be used.

Use fluid thioglycollate medium by incubating it at 30° to 35° under aerobic conditions.

Alternative thioglycollate medium – For use with turbnid and viscid products and for devices having tubes with small lumina.

$_L$-Cystine	0.5 g
Sodium chloride	2.5 g
Dextrose ($C_6H_{12}O_6,H_2O$)	5.5 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycollate or	0.5 g
Thioglycollic acid	0.3 ml
Distilled water to	1000 ml

Heat the ingredients in a suitable container until solution is effected. Mix and adjust the reaction with 1M sodium hydroxide, if necessary, so that the medium will have a pH of 7.1±0.2 after sterilization. Filter if necessary, place in suitable vessels and sterilise in an autoclave at 121° for 20 minutes.

The medium is freshly prepared or heated in a steam-bath and allowed to cool just prior to use. Do not reheat.

Use alternative thioglycollate medium in a manner that will assure anaerobic conditions for the duration of the incubation at 30° to 35°.

Soyabean-casein digest medium

Pancreatic digest of casein	17.0 g
Papaic digest of soyabean meal	3.0 g
Sodium chloride	5.0 g
Dibasic potassium phosphate (K_2HPO_4)	2.5 g
Dextrose ($C_6H_{12}O_6,H_2O$)	2.5 g
Distilled water to	1000 ml

Dissolve the solids in distilled water, warming slightly to effect solution. Cool to room temperature and add, if necessary, sufficient 0.1M sodium hydroxide to give a final pH of 7.1±0.2 after sterilisation. Fitler, if necessary, distribute into suitable containers and sterilize in an autoclave at 121º for 20 minutes.

Use soyabean-casein digest medium by incubating it at 20º to 25º under aerobic conditions.

Sterility : Incubate portions of the (a) fluid thioglycollate medium/alternate thioglycollate medium at 30º to 35º and (b) soyabean casein digest medium at 20º to 25º for not less than 7 days; no growth of micro-organisms occurs.

3.8.3. Growth promotion test :

Test each autoclaved load of each lot of the medium for its growth-promoting qualities by separately inoculating duplicate test containers of each medium with about 100 viable micro-organisms of each of the strains listed in Table 2 and incubating according to the conditions specified.

The test media are satisfactory if clear evidence of growth appears in all inoculated media containers within 7 days. The tests may be conducted simultaneously with the use of the test media for sterility test purposes. The sterility test is considered invalid if the test medium shows inadequate growth response.

If freshly prepared media are not used within 2 days, store them in dark, preferably at 2º to 25º.

Finished media, if stored in unsealed containers, may be used for not more than one month provided that they are tested within one week of the time of use.

3.8.4. Tests for bacteriostasis and fungistasis:

Prepare cultures of bacteria and fungi from the strain of micro-organisms mentioned in Table 2. Inoculate the sterility test media with about 100 viable micro-organisms using volumes of medium listed for liquids in Table 3. Add the specified portion of the preparation being examined to half of a suitable number of the containers already containing the inoculum and culture medium. Incubate the containers at the appropriate temperatures and under the conditions listed in Table 2 for not less than 7 days.

If growth of the test organisms in the preparation-medium mixture is visually co parable to that in the control vessels, use amounts of the preparation and medium regularly specified in Table 3.

If the preparation is bacteriostatic and/or fungistatic when tested as described above, use a suitable sterile neutralizing agent, if available. Suitability of such an agent is determined as in the test described below. If a neutralizing agent is not available, establish, as described below, suitable amounts of the preparation and medium to be used.

Repeat the tests set forth above, using the specified amount of the preparation and larger volumes of the medium to determine the ratio of the preparation to medium in which the growth of the test organisms is not adversely affected.

Table 2

Medium	Test micro-organisms	Incubation	
		Temperature (°)	Conditions
Fluid Thioglycollate	1. *Bacillus subtilis* (ATCC[1] No. 6633)[4] (NCIMB[2] No.8054)	30 to 35	Aerobic
	2. *Candida albicans* (ATCC No.10231) (NCYC[3] No.854)	30 to 35	Aerobic
	3. *Bacteroides vulgatus* (ATCC No.8482)[5]	30 to 35	Aerobic
Alternative Thioglycollate	1. *Bacteroides vulgatus* (ATCC No.8482)[5]	30 to 35	Anaerobic
Soyabean-Casein Digest	1. *Bacillus subtilis* (ATCC No. 6633)[4] 2. *Candida albicans* (ATCC No.10231)	20 to 25 20 to 25	Aerobic Aerobic

Note – Seed lot culture maintenance techniques should be employed so that the viable micro-organisms used for inoculation are not more than 5 passages removed from the Type cultures used.

Table 3 – Tests for bacteriostasis and fungistasis

Container content	*Minimum quantity of product*	*Minimum volume of culture medium (ml)*
For liquids		
Less than 1 ml	Total contents of a container	15
1 ml or more but less than 5 ml	Half the contents of a container	20
5 ml or more but less than 20 ml	2 ml	20
20 ml or more but less than 50 ml	5 ml	40
50 ml or more but less than 100 ml	10 ml	80
For solids		
Less than 50 mg	Total contents of a container	40
50 mg or more but less than 200 mg	Half the contents of a container	80
200 mg or more	100 mg	80

If the specified amount of the preparation is bacteriostatic or fungistatic in the medium, decrease the amount of the preparation to find the maximum amount that does not adversely affect the growth of the test organism in the medium. For liquids and suspensions, if this amount is less than 1 ml, increase the quantity of the medium so that the 1 ml is sufficiently diluted to prevent inhibition of growth. For solids that are not readily soluble or dispersible, if the amount is less than 50 mg, increase the quantity of the medium so that the 50 mg of the substance being examined is sufficiently diluted to prevent inhibition of growth. In either case, use the amount of the preparation and medium established in this ratio for sterility testing. Where membrane filtration is used, make similar comparisons using the specified portions of the preparation being examined and similar quantities of a suitable diluting and rinsing fluid, rinsing the membrane in each case with three portions, each of 100 ml of the diluting and rinsing fluid. Inoculate the stated quantities of viable micro-organisms into each final portion of the diluting and rinsing fluid used to filter the preparation being examined and filter the diluting and rinsing fluid only. The growth of the test organism in each case of the membrane (s) used to filter the preparation being examined followed by the inoculated final diluting and rinsing fluid is visually comparable to that from the membrane (s) used to filter only the inoculated diluting and rinsing fluid.

3.8.5. Test Procedures

The tests can be carried out using Method A, Membrane Filtration or Method B, Direct Inoculation. Method A is to be preferred where the substance being examined is (a) an oil, (b) an ointment that can be put into solution, (c) a non-bacteriostatic solid not readily soluble in the culture medium and (d) a soluble powder or a liquid that possesses inherent bacteriostatic and fungistatic properties.

For liquid products where the volume in a container is 100 ml or more, only Method A should be employed.

Select the number of samples to be tested from Table 1 and use them for the culture medium for bacteria and the culture medium for fungi.

Precautions : The tests for sterility should be carried out under conditions designed to avoid accidental contamination of the product during the test using, for example, a laminar sterile airflow cabinet. The precautions taken to avoid contamination must be such that they do not affect any micro-organisms that should be revealed in the test.

The working conditions in which the test is performed should be monitored regularly by sampling the air and surfaces of the working area and by carrying out control tests.

General Procedure : The exterior surface of ampoules and closures of vials and bottles should be cleaned with a suitable antimicrobial agent and access to the contents should be gained in a suitable aseptic manner. If the contents are packed in a container under vacuum, sterile air should be admitted by means of a suitable sterile device, such as a needle attached to a syringe barrel filled with non-absorbent cotton.

3.8.6. Method A : Membrane Filtration

The method needs exceptional skill and special knowledge; it also calls for the routine use of positive and negative controls. A suitable positive control is the occasional use of a known contaminated solution containing a few micro-organisms of different types (approximately 10 microbial cells in the total volumes employed).

(a) Apparatus

A suitable unit consists of a closed reservoir and a receptacle between which a properly supported membrance of appropriate porosity is placed. A membrane generally suitable for sterility testing has a nominal pore size not greater than 0.45 µm and diameter of approximately 47 mm, the effectiveness of which in retaining micro-organisms has been established. Preferably assemble and sterilize the entire unit with the membrane in place prior to use. Where the sample to be tested is an oil, sterilize the membrane separately and, after thorough drying, assemble the unit, using aseptic precautions.

Diluting fluids

Fluid A : Dissolve 1 g of peptic digest of animal tissue (such as bacteriological peptone) or its equivalent in water to make 1 litre, filter or centrifuge to clarify, adjust to pH 7.1±0.2, dispense into flasks in 100-ml quantities and sterilize at 121º for 20 minutes.

Fluid B : If the test sample contains lecithin or oil, use fluid A to each litre of which has been added 1 ml of polysorbate 80, adjust to pH 7.1±0.2, dispense into flasks and sterilize at 121º for 20 minutes.

Note – A sterile fluid shall not have antibacterial or antifungal properties if it is to be considered suitable for dissolving, diluting or rinsing a preparation being examined for sterility.

3.8.7. Method of Test

3.8.7.1. For aqueous solutions : Prepare each membrane by aseptically transferring a small quantity (sufficient to moisten the membrane) of fluid A on to the membrane and filtering it. For each medium to be used, transfer aseptically into two separate membrane filter funnels or to separate sterile pooling vessels prior to transfer not less than the quantity of the preparation being examined that is prescribed in Table 4 or Table 5. Alternatively, transfer aseptically the combined quantities of the preparation being examined prescribed in the two media onto one membrane. Draw the liquid rapidly through the filter with the aid of vacuum. If the solution being examined has antimicrobial properties, wash the membrane (s) by filtering through it (them) not less than three successive quantities, each of approximately 100 ml, of sterile fluid A. The quantities of fluid used should be sufficient to allow growth of a small inoculum of organisms (approximately 50) sensitive to the antimicrobial substance in the presence of the residual inhibitory material on the membrane.

After filtration, aseptically remove the membrane (s) from the holder, cut the membrane in half, if only one is used, immerse the membrane, or one-half of the

Table 4

Quantity in each container	*Minimum quantity to be used for each culture medium*
For liquids	
Less than 1 ml	Total contents of a container
1 ml or more but less than 4 ml	Half the contents of a container
4 ml or more but less than 20 ml	2 ml
20 ml or more but less than 100 ml	10% of the contents of a container unless otherwise specified in the monograph
100 ml or more	Not less than half the contents of a container unless otherwise specified in the monograph
For solids	
Less than 50 mg	Total contents of a container
50 mg or more but less than 200 mg	Half the contents of a container
200 mg or more	100 mg

Table 5

Type of preparation	*Quantity to be mixed (A)*	*Quantity to be used for each culture medium (B)*
Ophthalmic solutions; other non-injectable liquid preparations	10 to 100 ml	5 to 10 ml
Other preparations; preparations soluble in water or appropriate solvents; insoluble preparations to be suspended or emulsified (ointments and creams)	1 to 10 g	0.5 to 1 g
Absorbent cotton		Not less than 1 g*

*one portion

membrane, in 100 ml of soyabean-casein digest medium and incubate at 20º to 25º for not less than 7 days. Similarly, immerse the other membrane, or other half of the membrane, in 100 ml of fluid thioglycollate medium and incubate at 30º to 35º for not less than 7 days.

3.8.7.2. **For liquids immiscible with aqueous vehicles and suspensions** : Carry out the test described under **For aqueous solutions** but add a sufficient quantity of fluid A to the pooled sample to achieve rapid filtration. Sterile enzyme preparations such as

penicillinase or cellulase may be added to fluid A to aid in dissolving insoluble substances. If the substance under test contains lecithin, use fluid B for diluting.

3.8.7.3. For oils and oily solutions : Filter oils or oily solutions of sufficiently low viscosity without dilution thorough a dry membrane. Dilute viscous oils as necessary with a suitable sterile diluent such as isopropyl myristate that has been shown not to have antimicrobial properties under the conditions of test. Allow the oil to penetrate the membrane and filter by, applying pressure or suction gradually. Wash the membrane by filtering through it at least three successive quantities, each of approximately 100 ml, of sterile fluid B or any other suitable sterile diluent. Complete the test described under For aqueous solutions beginning at the words "After filtration,...".

3.8.7.4. For ointments and creams : Dilute ointments in a fatty base and emulsions of the water-in-oil type to give a fluid concentration of 1% w/v, by heating, if necessary, to not more than 40° with a suitable sterile diluent such as isopropyl myristate previously rendered sterile by filtration through a 0.22 µm membrane filter that has been shown not to have antimicrobial properties under the conditions of test. Filter as rapidly as possible and complete the test as described under For oils and oily solutions beginning at the words "Wash the membrane by ...". In exceptional cases, it may be necessary to heat the substance to not more than 45° and to use warm solutions for washing the membrane.

Note – For ointments and oils that are insoluble in isopropyl myristate, use Method B.

3.8.7.5. For soluble solids : For each medium, dissolve not less than the quantity of the substance being examined, as prescribed in Tables 4 and 5, in a suitable sterile solvent such as fluid A and carry out the test described under For aqueous solutions using a membrane appropriate to the chosen solvents.

3.8.7.6. For sterile devices : Aseptically pass a sufficient volume of fluid B through each of not less than twenty devices so that not less than 100 ml is recovered from each device. Collect the fluids in sterile containers and filter the entire volume collected through membrance filter funnel (s) as described under For aqueous solutions.

3.8.8. Method B: Direct Inoculation

Quantities of sample to be used

The quantity of the substance or preparation being examined which is to be used for inoculation in the culture media varies according to the quantity in each container and is given in Table 3 along with the volume of medium to be used.

(b) Method of test

3.8.8.1. For aqueous solutions and suspensions : The tests for microbial contamination are carried out on the same sample of the preparations being examined using the above-stated media. When the quantity in a single container is insufficient to carry out the tests, the combined contents of the two or more containers are used to inoculate the above-stated media.

Remove the liquid from the test containers with a sterile pipette or with a sterile

syringe or a needle. Aseptically transfer the specified volume of the material from each container to a vessel of the culture medium. Mix the liquid with the medium but do not aerate excessively. Incubate the inoculated media for not less than 14 days, unless otherwise specified in the monograph, at 30º to 35º in the case of fluid thioglycollate medium and at 20º to 25º in the case of soyabean-casein digest medium.

When the material being examined renders the medium turbid so that the presence or absence of microbial growth cannot be determined readily by visual examination, transfer suitable portions of the medium to fresh vessels of the same medium between the third and seventh days after the test is started. Continue incubation of the transfer vessels for not less than 7 additional days after the transfer and for a total of not less than 14 days.

3.8.8.2. For oils and oily solutions : Use media to which have been added 0.1% w/v of (4-tert-octylphenoxy) polyethoxyethanol, 1% w/v of polysorbate 80 or other suitable emulsifying agent, in an appropriate concentration, shown not to have any antimicrobial properties under the conditions of test. Carry out the test as described under For aqueous solutions and suspensions.

Cultures containing oily preparations should be shaken gently each day. However, when fluid thioglycollate medium is used for the detection of anaerobic micro-organisms, shaking or mixing should be kept to a minimum to maintain anaerobic conditions.

3.8.8.3. For ointments : Prepare by diluting ten-fold in a sterile diluent such as fluid B or any other aqueous vehicle capable of dispersing the test material homogeneously throughout the fluid mixture (Before use, test the dispersing agent to ascertain that in the concentration used it has no significant antimicrobial effects during the time interval for all transfers). Mix 10 ml of the fluid mixture so obtained with 80 ml of the medium and proceed as directed under For aqueous solutions and suspensions.

3.8.8.4. For solids: Transfer the quantity of the preparation to be examined to the quantity of medium specified in Table 5 and mix, the conditions of incubation being the same as For aqueous solutions and suspensions. Proceed as directed under For aqueous solutions and suspensions beginning at the words "When the material being examined...".

3.8.8.5. For sterile devices : For articles of such size and shape as permit complete immersion in not more than 1000 ml of culture medium test the intact article, using the appropriate media, and incubating as directed under For aqueous solutions and suspensions.

For transfusion or infusion assemblies or where the size of an item makes immersion impracticable and only the liquid pathway must be sterile, flush the lumen of each of twenty units with a sufficient quantity of fluid thioglycollate medium and the lumen of each of twenty units with a sufficient quantity of soyabean-casein digest medium to yield a recovery of not less than 15 ml of each medium, and incubate with not less than 100 ml of each of the two media as directed under For aqueous solutions and suspensions. For devices in which the lumen is so small that fluid thioglycollate medium will not pass through, substitute alternative thioglycollate medium for the fluid thioglycollate medium and incubate that inoculated medium anaerobically.

Where the presence of the specimen being tested, in the medium interferes with

the test because of bacteriostatic or fungistatic action, rinse the article thoroughly with the minimum amount of fluid A. Recover the rinsed fluid and test as described **For sterile devices** under Method A.

3.8.9. Observation and Interpretation of Results

At intervals during the incubation period, and at its conclusion, examine the media for macroscopic evidence of microbial growth. If no evidence of growth is found, the preparation being examined passes the tests for sterility. If evidence of microbial growth is found, reserve the containers showing this and, unless it is demonstrated by any other means that their presence is due to causes unrelated to the preparation being examined and hence that the tests for sterility are invalid and may therefore be recommenced, perform a retest using the same number of samples, volumes to be tested and the media as in the original test. If no evidence of microbial growth is then found, the preparation being examined passes the tests for sterility. If evidence of microbial growth is found, isolate and identify the organisms. If they are not readily distinguishable from those growing in the containers reserved in the first test, the preparation being examined fails the tests for sterility. If they are readily distinguishable from those growing in the containers reserved in the first test, perform a second retest using twice the number of samples. If no evidence of microbial growth is found in the second retest, the preparation being examined passes the tests for sterility. If evidence of growth of any micro-organisms is found in the second retest the preparation being examined fails the tests for sterility.

APPENDIX – 4

4.1 REAGENTS AND SOLUTIONS

Acetic Acid – Contains approximately 33 per cent w/v of $C_2H_4O_2$. Dilute 315 ml of glacial acetic acid to 1000 ml with *water*.

Acetic Acid, x N – Solutions of any normality xN may be prepared by diluting 60x ml of glacial acetic acid to 1000 ml with water.

Acetic Acid, Dilute – Contains approximately 6 per cent w/w of $C_2H_4O_2$. Dilute 57 ml of glacial acetic acid to 1000 ml with water.

Acetic Acid, Glacial – CH_3COOH =60.05.

Contains not less than 99.0 per cent w/w of $C_2H_4O_2$. About 17.5 N in strength.

Description – At temperature above its freezing point a clear colourless liquid, odour, pungent and characteristic; crystallises when cooled to about 10° and does not completely re-melt until warmed to about 15°.

Solubility – Miscible with *water,* with *glycerin* and most fixed and volatile oils.

Boiling range –Between 117° and 119°.

Congealing temperature –Not lower than 14.8°.

Wt. per ml –At 25° about 1.047 g.

Heavy metals –Evaporate 5 ml to dryness in a porcelain dish on water-bath, warm the residue with 2 ml of 0.1 *N hydrochloric acid* and water to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 2.3.3.

Chloride –5 ml complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –5 ml complies with the limit test for sulphates, Appendix 2.3.7.

Certain aldehydic substances – To 5 ml add 10 ml of *mercuric chloride solution* and make alkaline with *sodium hydroxide solution,* allow to stand for five minutes and acidify with dilute *sulphuric acid*; the solution does not show more than a faint turbidity.

Formic acid and oxidisable impurities – Dilute 5 ml with 10 ml of water, to 5 ml of this solution add 2.0 ml of 0.1 *N* potassium dichromate and 6 ml of sulphuric acid, and allow to stand for one minute, add 25 ml of water, cool to 15°, and add 1 ml of freshly prepared potassium iodide solution and titrate the liberated iodine with 0.1 *N* sodium thiosulphate, using starch solution as indicator. Not less than 1 ml of 0.*N sodium thiosulphate* is required.

Odorous impurities –Neutralise 1.5 ml with *sodium hydroxide solution*; the solution has no odour other than a faint acetous odour.

Readily oxidisable impurities – To 5 ml of the solution prepared for the test for Formic Acid and Oxidisable Impurities, add 20 ml of water and 0.5 ml of 0.1 *N potassium permanganate;* the pink colour does not entirely disappear within half a minute.

Non-volatile matter – Leaves not more than 0.01 per cent w/w of residue when evaporated to dryness and dried to constant weight at 105º.

Assay –Weigh accurately about 1 g into a stoppered flask containing 50 ml of *water* and titrate with *N sodium hydroxide,* using *phenolphthalein solution* as indicator. Each ml of *sodium hydroxide* is equivalent to 0.06005 g of $C_2H_4O_2$.

Acetic Acid, Lead-Free –Acetic acid which complies with following additional test, boil 25 ml until the volume is reduced to about 15 ml, cool make alkaline with lead-free ammonia solution, add 1 ml of lead free *potassium cyanide solution, dilute* to 50 ml with water, add 2 drops of *sodium sulphide solution;* no darkening is produced.

Acetone – Propan 2-one; $(CH_3)_2CO$ = 58.08

Description – Clear, colourless, mobile and volatile liquid; taste, pungent and sweetish; odour characteristic; flammable.

Solubility –Miscible with *water*, with alcohol, with *solvent ether,* and with *chloroform,* forming clear solutions.

Distillation range – Not less than 96.0 per cent distils between 55.5º and 57º.

Acidity– 10 ml diluted with 10 ml of freshly boiled and cooled water; does not require for neutralisation more than 0.2 ml of 0.1 *N sodium hydroxide*, using phenolphthalein solution as indicator.

Alkalinty – 10 ml diluted with 10 ml of freshly boiled and cooled water, is not alkaline to litmus solution.

Methyl alcohol –Dilute 10 ml with water to 100 ml. To 1 ml of the solution add 1 ml of *water* and 2 ml of *potassium permanganate* and *phosphoric acid solution*. Allow to stand for ten minutes and add 2 ml of *oxalic acid* and *sulphuric acid solution;* to the colourless solution add 5 ml of *decolorised magenta solution* and set aside for thirty minutes between 15º and 30º; no colour is produced.

Oxidisable substances –To 20 ml add 0.1 ml of 0.1 *N potassium permanganate,* and allow to stand for fifteen minutes; the solution is not completely decolorised.

Water – Shake 10 ml with 40 ml of *carbon disulphide*; a clear solution is produced.

Non-volatile matter –When evaporated on a water-bath and dried to constant weight at 105º, leaves not more than 0.01 per cent w/v residue.

Acetone Solution, Standard – A 0.05 per cent v/v solution of acetone in water.

Alcohol –

Description –Clear, colourless, mobile, volatile liquid, odour, characteristic and spirituous; taste, burning, readily volatilised even at low temperature, and boils at about 78º, flammable. Alcohol containing not less than 94.85 per cent v/v and not more than 95.2 per cent v/v of C_2H_5OH at 15.56º.

Solubility –Miscible in all proportions with *water,* with *chloroform* and with *solvent ether.*

Acidity or alkalinity – To 20 ml add five drops of *phenolphthalein solution*; the solution remains colourless and requires not more than 2.0 ml of 0.1*N sodium hydroxide* to produce a pink colour.

Specific gravity –Between 0.8084 and 0.8104 at 25º.

Clarity of solution –Dilute 5 ml to 100 ml with *water* in glass cylinder; the solution remains clear when examined against a black background. Cool to 10º for thirty minutes; the solution remains clear.

Methanol – To one drop add one of water, one drop of *dilute phosphoric acid,* and one drop of *potassium permanganate solution.* Mix, allow to stand for one minute and add sodium bisulphite solution dropwise, until the permanganate colour is discharged. If a brown colour remains, add one drop of *dilute phosphoric* acid. To the colourless solution add 5 ml of freshly prepared *chromotropic acid* solution and heat on a water-bath at 60º for ten minutes; no violet colour is produced.

Foreign organic substances – Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid,* rinse with water and finally rinse with the alcohol under examination. Put 20 ml in the cylinder, cool to about 15º and then add from a carefully cleaned pipette 0.1 ml 0.1 *N potassium permanganate.* Mix at once by inverting the stoppered cylinder and allow to stand at 15º for five minutes; the pink colour does not entirely disappear.

Isopropyl alcohol and t-butyl alcohol – To 1 ml add 2 ml of water and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within three minutes.

Aldehydes and ketones – Heat 100 ml of *hydroxylamine hydrochloride solution* in a loosely stoppered flask on a water-bath for thirty minutes, cool, and if necessary, add sufficient 0.05 *N sodium hydroxide* to restore the green colour. To 50 ml of this solution add 25 ml of the alcohol and heat on a water bath for ten minutes in a loosely stoppered flask. Cool, transfer to a Nesseler cylinder, and titrate with 0.05 *N sodium hydroxide* until the colour matches that of the remainder of the *hydroxylamine hydrochloride solution* contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.9 ml of 0.05 *N sodium hydroxide* is required.

Fusel oil constituents – Mix 10 ml with 5 ml of *water* and 1 ml of *glycerin* and allow the mixture to evaporate spontaneously from clean, odourless absorbent paper; no foreign odour is perceptible at any stage of the evaporation.

Non-volatile matter – Evaporate 40 ml in a tared dish on a water-bath and dry the residue at 105º for one hour; the weight of the residue does not exceed 1 mg.

Storage – Store in tightly-closed containers, away from fire.

Labelling – The label on the container states "Flammable".

Dilute Alcohols : Alcohol diluted with water to produce dilute alcohols. They are prepared as described below :

Alcohol (90 per cent)
Dilute 947 ml of alcohol to 1000 ml with water.

Specific Gravity –At 15.56º/15.56º, 0.832 to 0.835.

Alcohol (80 per cent)
Dilute 842 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.863 to 0.865,

Alcohol (60 per cent)
Dilute 623 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.913 to 0.914,

Alcohol (50 per cent)
Dilute 526 ml of alcohol to 1000 ml with water
Specific Gravity –At 15.56º/15.56º, 0.934 to 0.935.

Alcohol (25 per cent)
Dilute 263 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.9705 to 0.9713.

Alcohol (20 per cent)
Dilute 210 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.975 to 0976.

Alcohol, Aldehyde-free. – Alcohol which complies with the following additional test:

Aldehyde – To 25 ml, contained in 300 ml flask, add 75 ml of *dinitrophenyl hydrazine solution,* heat on a water bath under a reflux condenser for twenty four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of sulphuric acid, and set aside for twenty four hours; no crystals are produced.

Alcohol, Sulphate-free. – Shake alcohol with an excess of anion exchange resin for thirty minutes and filter.

Ammonia, xN. – Solutions of any normality xN may be prepared by diluting 75 x ml of strong ammonia solution to 1000 ml with water.

Ammonia-Ammonium Chloride Solution, Strong. – Dissolve 67.5 g of *ammonium chloride* in 710 ml of strong *ammonia solution* and add sufficient *water* to produce 1000 ml.

Ammonia Solution, Dilute. – Contains approximately 10 per cent w/w of NH_3.

Dilute 425 ml of *strong ammonia solution* to 1000 ml with *water*.

Wt. per ml – At 25º, about 0.960 g.

Storage – Dilute ammonia solution should be kept in a well-closed container, in a cool place.

Ammonia Solution 2 per cent –Ammonia solution 2 per cent is the ammonia solution strong diluted with purified water to contain 2 per cent v/v of Ammonia solution strong.Ammonia Solution, Strong –**Contains 25.0 per cent w/w of NH3 (limit, 24.5 to 25.5). About 13.5 N in strength**.

Description –Clear, colourless liquid; odour, strongly pungent and characteristic.

Solubility –Miscible with *water* in all proportions.

Wt. per. ml – At 25º, about 0.91g.

Heavy metals –Evaporate 5 ml to dryness on a water-bath. To the residue, add 1 ml of *dilute hydrochloric acid* and evaporate to dryness. Dissolve the residue in 2 ml of *dilute acetic acid* and add *water* to make 25 ml; the limit of heavy metals is 15 parts per million, Appendix 2.3.3.

Iron – Evaporate 40 ml on a water-bath to about 10 ml. The solution complies with the *limit test for iron*, Appendix 2.3.4

Chloride – Evaporate 40 ml on a water-bath to about 5 ml. The solution complies with the *limit test for chlorides*, Appendix 2.3.2.

Sulphate – Evaporate 20 ml on a water-bath to about 5 ml. The solution complies with *the limit test for sulphates*; Appendix 2.3.7.

Tarry matter – Dilute 5 ml with 10 ml of *water*, mix with 6 g of powdered *citric acid* in a small flask, and rotate until dissolved; no tarry or unpleasant odour is perceptible.

Non-volatile residue – Evaporate 50 ml to dryness in a tared porcelain dish and dry to constant weight at 105º, not more than 5 mg of residue remains.

Assay – Weigh accurately about 3 g in flask containing 50 ml of *N sulphuric acid* and titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.01703 g of NH_3.

Storage – Preserve strong Ammonia Solution in a well-closed container, in a cool place.

Ammonia Solution, Iron-free – Dilute ammonia solution which complies with the following additional test :-

Evaporate 5 ml nearly to dryness on a water-bath add 40 ml of water, 2 ml of 20 per cent w/v *solution of iron free citric acid* and 2 drops of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution and dilute* to 50 ml with *water*, no pink colour is produced.

Ammonia Buffer pH 10.00 – Ammonia buffer solution. Dissolve 5.4 g of *ammonium chloride* in 70 ml of 5 *N ammonia and dilute* with *water* to 100 ml.

Ammonium Chloride –NH_4Cl = 53.49

Description – Colourless crystals or white crystalline powder; odourless; taste, saline.

Solubility – Freely soluble in *water*, sparingly soluble in alcohol.

Arsenic – Not more than 4 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 10 parts per million, determined by method A, on 2.0 g dissolved in 25 ml of water, Appendix 2.3.3.

Barium – Dissolve 0.5 g in 10 ml of *water* and add 1 ml of *dilute sulphuric acid*; no turbidity is produced within two hours.

Sulphate – 2 g complies with the limit test for sulphates, Appendix 2.3.7

Thiocyanate – Acidify 10 ml of a 10 per cent w/v solution with *hydrochloric acid* and add a few drops of *ferric chloride solution*; no red colour is produced.

Sulphated ash – Not more than 0.1 per cent, Appendix 2.3.6.

Assay – Weigh accurately about 0.1 g, dissolve in 20 ml of water and add a mixture of 5 ml of *formaldehyde solution*, previously neutralised to *dilute phenolphthalein solution* and 20 ml of water. After two minutes, titrate slowly with 0.1 *N sodium hydroxide*, using a further 0.2 ml of *dilute phenolphthalein solution*. Each ml of 0.1N *sodium hydroxide* is equivalent to 0.005349 g of NH_4Cl.

Ammonium Chloride Solution –A 10.0 per cent w/v solution of *ammonium chloride* in water.

Ammonium Citrate Solution –Dissolve with cooling, 500 g *citric acid* in a mixture of 200 ml of *water* and 200 ml of 13.5 M ammonia, filter and dilute with water to 1000 ml.

Ammonium Nitrate – NH_4NO_3 =80.04

Description – Colourless crystals

Solubility – Freely soluble in water

Acidity – A solution in water is slightly acid to litmus *solution*.

Chloride – 3.5 g complies with the limit test for chloride, Appendix 2.3.2.

Sulphate – 5 g complies with the limit test for sulphates, Appendix 2.3.7.

Sulphated ash – Not more than 0.05 per cent, Appendix 2.3.6.

Ammonium Oxalate – $(CO_2NH_4)_2$. H_2O =142.11.

Description –Colourless crystals

Solubility – Soluble in water

Chloride –2 g, with an additional 20 ml of *dilute nitric acid,* complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –Dissolve 1 g in 50 ml of water, add 2.5 ml of hydrochloric acid and 1ml of *barium chloride solution* and allow to stand for one hour; no turbidity or precipitate is produced.

Sulphated ash – Not more than 0.005 percent, Appendix 2.3.6.

Ammonium Oxalate Solution –A 2.5 per cent w/v solution of *ammonium oxalate* in water.

Ammonium Phosphate – $(NH_4)_2HPO_4$ –

Description –White crystals or granules.

Solubility –Very soluble in wate; insoluble in alcohol.

Reaction – 1 g dissolved in 100 ml of *carbon dioxide-free water* has a reaction of about pH 8.0, using solution of cresol red as indicator.

Iron –2 g complies with the limit test for iron, Appendix 2.3.4.

Chloride – 2 g with an additional 3.5 ml of nitric acid complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –2.5 g with an additional 4ml of hydrochloric acid, complies with the limit test for sulphate, Appendix 2.3.2.

Ammonium Phosphate, Solution –A 10.0 per cent w/v solution of ammonium phosphate in water.

Ammonium Thiocyanate – NH_4SCN = 76.12.

Description –Colourless crystals.

Solubility – Very soluble in water, forming a clear solution, readily soluble in alcohol.

Chloride –Dissolve 1 g in 30 ml of solution of hydrogen peroxide, add 1 g of *sodium hydroxide*, warm gently, rotate the flask until a vigorous reaction commences and allow to stand until the reaction is complete; add a further 30 ml of *hydrogen peroxide solution* boil for two minutes, cool, and add 10 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; any opalescence produced is not greater than that obtained by treating 0.2 ml of 0.01 *N hydrochloric acid* in the same manner.

Sulphated ash –Moisten 1 g with *sulphuric acid* and ignite gently, again moisten with sulphuric acid and ignite; the residue weighs not more than 2.0 mg.

Ammonium Thiocyanate, 0.1N – NH_4SCN = 76.12; 7.612 in 1000 ml. Dissolve about 8 g of *ammonium thiocyanate* in 100o ml of water and standardise the solution as follows :

Pipette 30 ml of standardised 0.1 *N silver nitrate* into a glass stoppered flask, dilute with 50 ml of *water* then add 2 ml of *nitric acid* and 2 ml of *ferric ammonium sulphate solution* and titrate with the *ammonium thiocyanate solution* to the first appearance of a red brown colour. Each ml of 0.1N silver nitrate is equivalent to 0.007612 g of NH_4SCN.

Ammonium Thiocyanate Solution – A 10.0 per cent w/v solution of *ammonium thiocyanate solution*.

Anisaldehyde-Sulphuric Acid Reagent – 0.5 ml *anisaldehyde* is mixed with 10 ml *glacial acetic acid*, followed by 85 ml methanol and 5 ml concentrated *sulphuric acid* in that order.

The reagent has only limited stability and is no longer usable when the colour has turned to redviolet.

Arsenic Trioxide –As_2O_3 = 197.82. Contains not less than 99.8 per cent of As_2O_3.

Description – Heavy white powder

Solubility –Sparingly soluble in water; more readily soluble in water on the addition of *hydrochloric acid*, or solutions of alkali hydroxides or carbonates.

Arsenious sulphide – Weigh accurately 0.50 g and dissolve in 10 ml of *dilute ammonia sulution;* forms a clear colourless solution which, when diluted with an equal volume of water and acidified with *hydrochloric acid*, does not become yellow.

Non-volatile matter –Leaves not more than 0.1 per cent of residue when volatilised.

Assay –Weigh accurately about 0.2 g and dissolve in 20 ml of boiling water and 5 ml of *N sodium hydroxide*, cool, and 5 ml of *N hydrochloric acid* and 3 g of *sodium bicarbonate,* and titrate with 0.1 *N iodine.* Each ml of 0.1N iodine is equivalent to 0.004946 g of As_2O_3.

Barium Chloride - $BaCl_2$, $2H_2O$ =244.27.

Description – Colourless crystals.

Solubility –Freely soluble in water.

Lead –Dissolve 1 g in 40 ml of recently boiled and cooled water, add 5 ml of *lead free acetic acid*. Render alkaline with *lead-free ammonia solution* and add 2 drops of *lead-free sodium sulphide solution;* not more than a slight colour is produced.

Nitrate –Dissolve 1 g in 10 ml of *water*, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen free sulphuric acid* and heat to boiling; the blue colour does not entirely disappear.

Barium Chloride Solution –A 10.0 per cent w/v solution of *barium chloride* in *water*.

Bismuth Oxynitrate – Bismuth Oxide Nitrate, Contains 70.0 to 74.0 per cent of Bi.

Description –White, microcrystalline powder.

Solubility – Practically insoluble in water, in alcohol; freely soluble in *dilute nitric acid* and in *dilute hydrochloric acid*.

Assay – Weigh accurately about 1 g and dissolve in a mixture of 20 ml of *glycerin* and 20 ml of water. Add 0.1 g of s*ulphamic acid* and titrate with 0.05 M *disodium ethylenediamine tetraacetate*, using *catechol violet solution* as indicator. Each ml of 0.05 M disodium ethylenediamine tetra-acetate is equivalent to 0.01045 g of Bi.

Borax - Sodium Tetraborate, $Na_2B_4O_7$. $10H_2O$ = 381.37. Contains not less than 99.0 per cent and not more than the equivalent of 103.0 per cent of $Na_2B_4O_7$. $10H_2O$.

Description – Transparent, colourless crystals, or a white, crystalline powder; odourless, taste, saline and alkaline. Effloreces in dry air, and on ignition, loses all its water of crystallisation.

Solubility – Soluble in water, practically insoluble in alcohol.

Alkalinity – A solution is alkaline to litmus solution.

Heavy metals – Dissolve 1 g in 16 ml of water and 6 ml of *N hydrochloric acid* and add *water* to make 25 ml; the limit of heavy metals is 20 parts per million, Appendix 2.3.3.

Iron –0.5 g complies with the *limit test for iron*, Appendix 2.3.4

Chlorides –1 g complies with the *limit test for chlorides,* Appendix 2.3.2

Sulphates –1g complies with the *limit test for sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 3 g and dissolve in 75 ml of *water* and titrate with 0.5 *N hydrochloric acid*, using *methyl red solution* as indicator. Each ml of 0.5 *N* hydrochloric acid is equivalent to 0.09534 g of $Na_2B_4O_7.10.H_2O$.

Storage – Preserve Borax in well-closed container.

Boric Acid –H_3BO_3 = 61.83.

Description –Colourless plates or white crystals or white crystalline powder, greasy to touch; odourless; taste, slightly acid and bitter with a sweetish after taste.

Solubility –Soluble in *water* and in *alcohol*; freely soluble in boiling *water*, in boiling *alcohol* and in *glycerin.*

Sulphate –Boil 3 g with 30 ml of *water* and 1 ml of *hydrochloric acid*, cool, and filter; 25 ml of the filtrate complies with the *limit test for sulphates*, Appendix 2.3.7.

Arsenic –Not more than 10 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 20 parts per million, determined by Method A on a solution obtained by dissolving 1.0 g in 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, Appendix 2.3.3.

Assay –Weigh accurately about 2 g, and dissolve in a mixture of 50 ml of *water* and 100 ml of *glycerine,* previously neutralised to *phenolphthalein solution*. Titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.06183 g of H_3BO_3.

Storage –Store in well-closed containers.

Labelling –The label on the container states "Not for internal use".

Boric Acid Solution –Dissolve 5 g of boric acid in a mixture of 20 ml of *water* and 20 ml of *absolute ethanol* and dilute with *absolute ethanol* to 250 ml.

Bromine – Br2 =159.80.

Description –Reddish-brown, fuming, corrosive liquid.

Solubility –Slightly soluble in *water*, soluble in most organic solvents.

Iodine –Boil 0.2 ml with 20 ml of *water*, 0.2 ml of *N sulphuric acid* and a small piece of marble until the liquid is almost colourless. Cool, add one drop of *liquified phenol*, allow to stand for two minutes, and then add 0.2 g of *potassium iodide* and 1 ml of *starch solution*; no blue colour is produced.

Sulphate –Shake 3 ml with 30 ml of *dilute ammonia solution* and evaporate to dryness on a water bath, the residue complies with the *limit test for sulphates*, Appendix 2.3.7.

Bromine Solution – Dissolve 9.6 ml of *bromine* and 30 g of *potassium bromide* in sufficient *water* to produce 100 ml.

Bromocresol Purple – 4,4′ –(3H-2, 1-Benzoxathiol-3-ylidene) bis-(2,6-dibromo-o-cresol) SS-dioxide; $C_{21}H_{14}Br_2O_4S$ =540.2.

Gives a yellow colour in weakly acid solutions and a bluish-violet colour in alkaline, neutral and extremely weakly acid solutions (pH range, 5.2 to 6.8).

Bromocresol Purple Solution –Warm 0.1 g of *bromocresol purple* with 5 ml of *ethanol* (90 per cent) until dissolved, add 100 ml of ethanol (20 per cent), 3.7 ml of 0.05 M s*odium hydroxide*, and sufficient *ethanol (20 per cent)* to produce 250 ml.

Complies with the following test :

Sensitivity – A mixture of 0.2 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.05 ml of 0.02 M *sodium hydroxide* has been added is bluish-violet. Not more than 0.20 ml of 0.02 M *hydrochloric acid is* required to change the colour to yellow.

Bromophenol Blue – 4, 4/, - (3H-2, 1 – Benzoxathiol - 3-ylidene) bis- (2-6-dibromophenol) SS-dioxide $C_{19}H_{19}Br_4O_5S$ = 670.

Gives a yellow colour in moderately acid solutions, and a bluish-violet colour in weakly acid and alkaline solutions (pH range, 2.8 to 4.6).

Bromophenol Blue Solution – Warm 0.1 g of *bromophenol blue* with 3.0 ml of 0.05 *N sodium hydroxide* and 5 ml of *alcohol* (90 per cent); after solution is effected, add sufficient *alcohol* (20 per cent) to produce 250 ml.

Complies with the following test :

Sensitivity –A mixture of 0.05 ml of the solution and 20 ml of *carbon dioxide-free water* to which 0.05 ml of 0.1*N hydrochloric acid* has been added is yellow. Not more than 0.10 ml of 0.1 *N sodium hydroxide* is required to change the colour to bluish-violet.

Bromothymol Blue –6, 6/–(3H-2, 1–Benzoxathiol–3–ylidene) bis –(2 – bromothymol) SS–dioxide $C_{27}H_{28}Br_2O_5S$ = 624.

Gives a yellow colour in weakly acid solutions and a blue colour in weakly alkaline solutions. Neutrality is indicated by a green colour (pH range, 6.0 to 7.6).

Bromothymol Blue Solution –Warm 0.1 g of *bromothymol blue* with 3.2 ml of 0.05 *N sodium hydroxide* and 5 ml of alcohol (90 per cent); after solution is effected, add sufficient alcohol (20 per cent) to produce 250 ml.

Complies with the following test :

Sensitivity –A mixture to 0.3 ml of the solution and 100 ml of c*arbon dioxide-free water* is yellow. Not more than 0.10 ml of 0.02 *N sodium hydroxide* is required to change the colour to blue.

Cadmium Iodide – CdI_2 = 366.23

Description –Pearly white flakes or a crystalline powder.

Solubility –Freely soluble in water.

Iodate –Dissolve 0.2 g in 10 ml of *water*, and add 0.5 g of *citric acid* and 1 ml of *starch solution*, no blue colour is produced.

Cadmium Iodide Solution – A 5.0 per cent w/v solution of *cadmium iodide* in *water*.

Calcium Carbonate – $CaCO_3$ = 100 .1

Analytical reagent grade of commerce.

Calcium Chloride – $CaCl_2$ H_2O = 147.0.

Analytical reagent grade of commerce.

Calcium Chloride Solution –A 10 per cent w/v solution of calcium chloride in water.

Calcium Hydroxide – $Ca(OH)_2$ = 74.09

Analytical reagent grade of commerce.

Calcium Hydroxide Solution –Shake 10 g of calcium hydroxide repeatedly with 1000 ml of water and allow to stand until clear.

Calcium Sulphate – $CaSO_4$, $2H_2O$ = 172.17.

Description –White powder.

Solubility –Slightly soluble in *water*.

Chloride –Boil 5 g with 50 ml of *water* and filter while hot. The filtrate, after cooling complies with the limit test for chlorides, Appendix 2.3.2.

Acid-insoluble matter –Boil 2 g with 100 ml of *N hydrochloric acid*; and then with *water*, dry, ignite, and weigh; the residue weighs not more than 2 mg.

Alkalinity –Boil 1 g with 50 ml of water, cool, and titrate with 0.*1 N hydrochloric acid*, *using bromo thymol blue solution* as indicator; not more than 0.3 ml of 0.1 *N hydrochloric acid* is required.

Carbonate –Boil 1 g with 10 ml of *water* and 1 ml of *hydrochloric acid,* no carbon dioxide is evolved.

Residue on ignition –When ignited, leaves not less than 78.5 per cent and not more than 80.0 per cent of residue.

Camphor –**$C_{10}H_{16}O$ = 152.23**

Camphor is a ketone, obtained from *Cinnamomum camphora* (Linn.) Nees and Eberm. (Fam. Lauraceae) and *Ocimum kilimandscharicum Guerke* (Fam. Labiatae) (Natural Camphor) or produced synthetically (Synthetic Camphor). It contains not less than 96.0 per cent of $C_{10}H_{16}O$.

Description – Colourless or white crystals, granules or crystalline masses or colourless to white, translucent tough masses; odour, penetrating and characteristic; taste, pungent, aromatic, and followed by a sensation of cold. Readily pulverisable in the presence of a little *alcohol, chloroform*, or solvent ether.

Solubility –Slightly soluble in water, very soluble in *alcohol, in chloroform* and in *solvent ether*, freely soluble in fixed oils and in volatile oils.

Melting range –174° to 179°.

Specific optical rotation – + 41° to + 43°, determined in a 10 per cent w/v solution of Natural Camphor in alcohol. Synthetic Camphor is the optically inactive, racemic form.

Water – A 10 per cent w/v solution in light petroleum (boiling range 40º to 60º) is clear.

Non-volatile matter – Leaves not more than 0.05 per cent of residue when volatilised at 105º.

Assay – Weigh accurately about 0.2 g and dissolve in 25 ml of *aldehyde-free alcohol*, in a 300-ml flask. Slowly add while stirring 75 ml of *dinitrophenylhydrazine* solution and heat on a water-bath for four hours under a reflux condenser. Remove the alcohol by distillation, allow to cool, dilute to 200 ml with a 2 per cent v/v solution of *sulphuric acid* in water. Set aside for twenty-four hours, filter in a tared Gooch crucible, and wash the precipitate with successive quantities of 10 ml of cold water until the washings are neutral to *litmus paper*. Dry to constant weight at 80º and weigh. Each g of precipitate is equivalent to 0.458 g of $C_{10}H_{16}O$.

Storage –Preserve Camphor in a well-closed container in a cool place.

Canada Balsam Reagent –General reagent grade of commerce.

Carbon Dioxide – CO_2 = 44. 01.

Commercially available carbon dioxide.

Carbon Disulphide – CS_2 = 76.14

Description –Clear, almost colourless, flammable liquid.

Distillation range – Not less than 95 per cent distils between 46º and 47º.

Wt. per ml – At 25º, about 1.263 g.

Non-volatile matter – When evaporated to dryness on a water bath, and dried to constant weight at 105º. Leaves not more than 0.005 per cent w/v of residue.

Carbon Tetrachloride – CCl_4 = 153.82

Description –Clear, colourless, volatile, liquid; odour, characteristic.

Solubility –Practically insoluble in water; miscible with ethyl alcohol, and with solvent ether.

Distillation range –Not less than 95 per cent distils between 76º and 77º.

Wt per ml – At 20º, 1.592 to 1.595 g.

Chloride, Free acid –Shake 20 ml with 20 ml of freshly boiled and cooled water for three minutes and allow separation to take place; the aqueous layer complies with the following test :

Chloride – To 10 ml add one drop of nitric acid and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free acid –To 10 ml add a few drops of *bromocresol purple solution;* the colour produced does not indicate more acidity than that indicated by the addition of the same quantity of the indicator to 10 ml of freshly boiled and cooled *water.*

Free chlorine –Shake 10 ml with 5 ml of *cadmium iodide solution* and 1 ml *of starch solution*, no blue colour is produced.

Oxidisable impurities – Shake 20 ml for five minutes with a cold mixture of 10 ml of *sulphuric acid* and 10 ml of 0.1 *N potassium dichromate*, dilute with 100 ml of water and add 3 g of *potassium iodide* : the liberated iodine requires for decolourisation not less than 9 ml of 0.1 *N sodium thiosulphate*.

Non-volatile matter – Leaves on evaporation on a water-bath and drying to constant weight at 105° not more than 0.002 per cent w/v of residue.

Caustic Alkali Solution, 5 per cent –

Dissolve 5 g of *potassium or sodium hydroxide* in *water* and dilute to 100 ml.

Charcoal, Decolourising – General purpose grade complying with the following test.

Decolourising powder – Add 0.10 g to 50 ml of 0.006 per cent w/v solution of *bromophenol blue* in ethanol (20 per cent) contained in a 250 ml flask, and mix. Allow to stand for five minutes, and filter; the colour of the filterate is not deeper than that of a solution prepared by diluting 1 ml of the *bromophenol blue solution* with *ethanol* (20 per cent) to 50 ml.

Chloral Hydrate – $CCl_3.CH(OH)_2$ = 165.40.

Description – Colourless, transparent crystals, odour, pungent but not acrid; taste, pungent and slightly bitter, volatilises slowly on exposure to air.

Solubility – Very soluble in *water,* freely soluble in *alcohol*, in chloroform and in *solvent ether*.

Chloral alcoholate – Warm 1 g with 6 ml of *water* and 0.5 ml of *sodium hydroxide solution* : filter, add sufficient 0.*1 N iodine* to impart a deep brown colour, and set aside for one hour; no yellow crystalline precipitate is produced and no smell of iodoform is perceptible.

Chloride – 3 g complies with the limit test for chlorides, Appendix 2.3.2.

Assay – Weigh accurately about 4 g and dissolve in 10 ml of *water* and add 30 ml of *N sodium hydroxide.* Allow the mixture to stand for two minutes, and then titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. Titrate the neutralised liquid with 0.1 *N silver nitrate* using solution of *potassium chromate* as indicator. Add two-fifteenth of the amount of 0.1 *N silver nitrate* used to the amount of *N sulphuric acid* used in the first titration and deduct the figure so obtained from the amount of *N sodium hydroxide* added. Each ml of *N sodium hydroxide,* obtained as difference; is equivalent to 0.1654 g of $C_2H_3Cl_3O_2$.

Storage – Store in tightly closed, light resistant containers in a cool place.

Chloral Hydrate Solution – Dissolve 20 g of *chloral hydrate* in 5 ml of water with warming and add 5 ml of *glycerin*.

Chloral Iodine Solution –Add an excess of crystalline *iodine with* shaking to the *chloral hydrate solution*, so that crystals of undissolved iodine remain on the bottom of bottle. Shake before use as the iodine dissolves, and crystals of the iodine to the solution. Store in a bottle of amber glass in a place protected from light.

Chlorinated Lime – Bleaching powder. Contains not less than 3.0 per cent of available chlorine.

Description – A dull white powder; odour characteristic. On exposure to air it becomes moist and gradually decomposes.

Solubility – Slightly soluble in water and in alcohol.

Stability – Loses not more than 3.0 per cent of its available chlorine by weight when heated to 100° for two hours (The available chlorine is determined by the Assay described below).

Assay – Weigh accurately about 4 g, triturate in a mortar with successive small quantities of water and transfer to a 1000 ml flask. Add sufficient water to produce 1000 ml and shake thoroughly. To 100 ml to this suspension add 3 g of *potassium iodide* dissolved in 100 ml of *water*, acidify with 5 ml of *acetic acid* and titrate the liberated iodine with 0.1 *N sodium thiosulphate*. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.003545 g of available chlorine.

Storage – Preserve in a well-closed container.

Chlorinated Lime Solution – Mix 100 g of *chlorinated lime* with 1000 ml of *water;* transfer the mixture to a stoppered bottle; set aside for three hours, shaking occasionally; filter through calico.

Chlorinated lime solution must be recently prepared.

Chloroform – $CHCl_3$ = 119.38

Description –Colourles, volatile liquid; odour, characteristic. Taste, sweet and burning.

Solubility –Slightly soluble in water; freely miscible with ethyl alcohol and with solvent ether.

Wt. Per ml. : Between 1.474 and 1.478 g.

Boiling range – A variable fraction, not exceeding 5 per cent v/v, distils below 60° and the remainder distils between 50° to 62°.

Acidity –Shake 10 ml with 20 ml of freshly boiled and cooled water for three minutes, and allow to separate. To a 5 ml portion of the aqueous layer add 0.1 ml of *litmus solution*; the colour produced is not different from that produced on adding 0.1 ml of *litmus solution* to 5 ml of freshly boiled and cooled water.

Chloride –To another 5 ml portion of the aqueous layer obtained in the test for Acidity, add 5 ml of water and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free chlorine –To another 10 ml portion of the aqueous layer, obtained in the test for Acidity, add 1 ml of *cadmium iodide solution* and two drops of starch solution; no blue colour is produced.

Aldehyde –Shake 5 ml with 5 ml of water and 0.2 ml of *alkaline potassium mercuri-iodide solution* in a stoppered bottle and set aside in the dark for fifteen minutes; not more than a pale yellow colour is produced.

Decomposition products – Place 20 ml of the *chloroform* in a glass-stoppered flask, previously rinsed with *sulphuric acid,* add 15 ml of *sulphuric acid* and four drops of *formaldehyde solution*, and shake the mixture frequently during half an hour and set aside for further half an hour, the flask being protected from light during the test; the acid layer is not more than slightly coloured.

Foreign organic matter – Shake 20 ml with 10 ml of *sulphuric* acid in a stoppered vessel previously rinsed with *sulphuric acid* for five minutes and set aside in the dark for thirty minutes, both the acid and chloroform layers remain colourless. To 2 ml of the acid layer add 5 ml of water; the liquid remains colourless and clear, and has no unpleasent odour. Add a further 10 ml of water and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Foreign odour –Allow 10 ml to evaporate from a large piece of filter paper placed on a warm plate; no foreign odour is detectable at any stage of the evaporation.

Non volatile matter – Not more than 0.004 per cent w/v determined on 25 ml by evaporation and drying at 105^{o}.

Storage : Store in tightly-closed, glass-stoppered, light-resistant bottles.

Note:- Care should be taken not to vaporise Chloroform in the presence of a flame because of the production of harmful gases.

Chloroform Water –

Chloroform : 2.5 ml

Purified Water : sufficient to produce 1000 ml

Dissolve the C*hloroform* in the purified water by shaking.

Chromic-Sulphuric Acid Mixture –A saturated solution of Chromium trioxide in sulphuric acid.

Chromium Trioxide – CrO_3 = 99.99

Analytical reagent grade.

Chromotropic Acid – $C_{10}H_8O_8S_2.2H_2O$ = 356.32

Description –White to brownish powder. It is usually available as its sodium salt, $C_{10}H_8O_8S_2Na_2$, which is yellow to light brown in colour.

Solubility –Soluble in water; sodium salt is freely soluble in water.

Sensitivity –Dilute exactly 0.5 ml *formaldehyde solution* with water to make 1000 ml. Disslove 5 mg of *chromotropic acid* or its sodium salt, in a 10 ml of a mixture of 9 ml *of sulphuric acid* and 4 ml of water. Add 5 ml of this solution to 0.2 ml of the *formaldehyde solution*, and heat for 10 minutes at 60^{o}; a violet colour is produced.

Chromotropic Acid Solution –Dissolve 5 mg of *chromotropic acid sodium* salt in 10 ml of a mixture of 9 ml of *sulphuric acid* and 4 ml of water.

Citric Acid – $C_6H_8O_7$, H_2O =210.1

Colourless, translucent crystals, or a white, crystalline powder, slightly hygroscopic in moist air and slightly efflorescent in warm dry air; odourless; taste, strongly acid.

Analytical reagent grade.

Citric Acid, Iron-Free –Citric acid which complies following additional test :

Dissolve 0.5 g in 40 ml of water, add 2 drops of thioglycollic acid, mix, make alkaline with iron free ammonia solution and dilute to 50 ml with water; no pink colour is produced.

Copper Acetate –Cu $(C_2H_3O_2)_2$, H_2O =199.65

Contains not less than 98.0 per cent of $C_4H_6O_4Cu$, H_2O

Description –Blue-green crystals or powder, having a faint odour of acetic acid.

Solubility – Soluble in water, yielding a clear solution.

Chloride –3g complies with the *limit test for chlorides*, Appendix 2.3.2.

Sulphate –3g complies with the *limit test for sulphates*, Appendix 2.3.7.

Assay –Weigh accurately about 0.8 g and dissolve in 50 ml of water, add 2 ml of *acetic acid* and 3 g of *potassium iodide*, and titrate the liberated iodine with 0.1 *N sodium thiosulphate*, using starch solution as indicator, until only a faint blue colour remains; add 2 g of *potassium thiocyanate* and continue the titration until the blue colour disappears. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.01997 g of $C_4H_6O_4Cu$, H_2O

Copper Acetate, Solution –0.5 per cent w/v of copper acetate in water.

Copper Sulphate – $CuSO_4$, $5H_2O$ = 249.68

Contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of $CuSO_4$, $5H_2O$.

Description –Blue triclinic prisms or a blue, crystalline powder.

Solubility –Soluble in *water*, very solube in boiling water, almost insoluble in *alcohol*; very slowly soluble in glycerin.

Acidity and clarity of solution – 1 g, dissolved in 20 ml of water, forms a clear blue solution, which becomes green on the addition of 0.1 ml of *methyl orange solution*.

Iron – To 5 g, add 25 ml of water, and 2 ml of nitric acid, boil and cool. Add excess of *strong ammonia solution*, filter, and wash the residue with *dilute ammonia solution* mixed with four times its volumes of water. Dissolve the residue, if any, on the filter with 2 ml of *hydrochloric acid*, diluted with 10 ml of water; to the acid solutions add *dilute ammonia solution* till the precipitation is complete; filter and wash; the residue after ignition weighs not more than 7 mg.

Copper Sulphate, Anhydrous –$CuSO_4$ =159.6

Prepared by heating copper sulphate to constant weight at about 230º.

Copper Sulphate Solution –A10.0 per cent w/v solution of *copper sulphate* in *water*.

Catechol Violet – 4,4/ –(3H-2, I-Benzoxathiol-3-ylidene) diphyrocatechol SS-dioxide.

Gives a blue colour with bishmuth ions in moderately acid solution. When metal ions are absent, for example, in the presence of an excess of *disodium ethylenediamine tetra-acetate*, the solution is yellow.

Catechol Violet Solution – Dissolve 0.1 g of catechol violet in 100 ml of water.

Cresol Red – 4,4/, –(3H-2, 1-Benzoxathiol-3 ylidene) di-o-cresol SS-dioxide; $C_{12}H_8O_5S$ = 382.4.

Gives a red colour in very strongly acid solutions, a yellow colour in less strongly acid and neutral solutions, and a red colour in moderately alkaline solutions (pH ranges, 0.2 to 1.8, and 7.2 to 8.8).

Cresol Red Solution –Warm 50 ml of *cresol red* with 2.65 ml of 0.05 M s*odium hydroxide* and 5 ml of *ethanol (90 per cent);* after solution is effected, add sufficient ethanol (20 per cent) to produce 250 ml.

Sensitivity –A mixitue of 0.1 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.15 ml of 0.02 M s*odium hydroxide* has been added is purplish-red.Not more than 0.15 ml of 0.02 *M hydrochloric acid* is required to change the colour to yellow.

Dimethyl Yellow – 4 –Dimethyl aminoazobenzene ; $C_{14}H_{15}5N_3$ = 225.3

Gives a red colour in moderately acid alcoholic solutions, and a yellow colour in weakly acid and alkaline solution (pH range, 2.8 to 4.0).

Dimethyl Yellow Solution –A 0.2 per cent w/v solution of *dimethyl yellow* in alcohol (90 per cent).

Sensitivity – A solution containing 2 g of ammonium chloride in 25 ml of *carbon dioxide-free water,* to which is added 0.1 ml of the *dimethyl yellow solution*, is yellow. Not more than 0.10 ml of 0.1 *N hydrochloric acid* is required to change the colour to red.

Dinitrophenylhydrazine –2,4–Dinitrophenylhydrazine; $(NO_2)_2C_6H_3$, NH, NH_3 = 198.14.

Description –Orange-red crystals or a crystalline powder.

Solubility –Practically insoluble in *water,* slightly soluble in alcohol.

Clarity and colour of solution – 0.5 g yields a clear yellow solution on heating with a mixture of 25 ml of water and 25 ml of h*ydrochloric acid*.

Melting range –197° to 200°, with decomposition.

Sulphated ash –Not more than 0.5 per cent, Appendix 2.3.6.

Dinitrophenylhydrazine Solution –Dissolve 1.5 gm of *dinitrophenylhydrazine* in 20 ml of sulphuric acid (50 per cent v/v). Dilute to 100 ml with *water* and filter.

Dinitrophenylhydrazine solution must be freshly prepared.

Diphenylbenzidine –$(C_6H_5.\ NH.\ C_6H_4)_2$ = 336.42.

Description – White for faintly grey coloured, crystalline powder.

Melting range –246° to 250°.

Nitrate –Dissolve 8 mg in a cooled mixture of 45 ml of *nitrogen free sulphuric acid* and 5 ml of water; the solution is colourless or not more than very pale blue.

Sulphated ash –Not more than 0.1 per cent, Appendix 2.3.6.

Diphenylcarbazide –1,5-Diphenylcarbazide : $(C_6H_5NH.\ NH)_2\ CO$ = 242.27.

Description –White crystalline powder which gradually acquires a pink tint on exposure to air.

Solubility –Practically insoluble in *water*; soluble in alcohol.

Diphenylcarbazide Solution –A 0.2 per cent w/v solution of *diphenylcarbazide* in a mixture of 10 ml of glacial acetic acid and 90 ml of *alcohol (90 per cent).*

Diphenylthiocarbazone –Dithizone : 1,5–Diphenylthiocarbazone; C_6H_5N : NCS. NH. NH. C_6H_5 = 256.32.

Description –Almost black powder.

Solubility –Practically insoluble in *water*; soluble in *chloroform*, in carbon tetrachloride and in other organic solvents, yielding solutions of an intense green colour.

Lead –Shake 5 ml of 0.1 per cent w/v solution in *chloroform* with a mixture of 5 ml of *water*, 2 ml of *lead free potassium cyanide solution,* and 5 ml of *strong ammonia solution*; the chloroform layer may remain yellow but has no red tint.

Sulphated ash –Not more than 0.5 per cent, Appendix 2.3.6.

Disodium Ethylenediamine tetraacetate – (Disodium Acetate) $C_{10}H_{14}N_2Na_2O_8$, $2H_2O$ = 372.2

Analytical reagent grade.

Dragendorff Reagent –

Solution 1 –Dissolve 0.85 g of *bismuth oxy nitrate* in 40 ml of water and 10 ml of acetic acid.

Solution 2 –Dissolve 8 g of *potassium iodide* in 20 ml of water.

Mix equal volumes of solution 1 and 2, and to 10 ml of the resultant mixture add 100 ml of water and 20 ml of acetic acid.

Eosin – Acid Red 87; Tetrabromofluorescein disodium salt; $C_{20}H_6O_5Br_4Na_2$ =691.86.

Description – Red powder, dissolves in water to yield a yellow to *purplish-red* solution with a greenish-yellow fluorescence.

Solubility –Soluble in *water* and in alcohol.

Chloride –Dissolve 50 mg in 25 ml of *water*, add 1 ml of *nitric acid*, and filter; the filtrate complies with *the limit test for chlorides*, Appendix 2.3.2.

Sulphated ash –Not more than 24.0 per cent, calculated with reference to the substance dried at 110° for two hours, Appendix 2.3.6.

Eosin Solution –A 0.5 per cent w/v solution of eosin in water.

Eriochrome Black T –Mordant Black 11; Sodium 2(1-hydroxy-2-naphthylazo) 5-nitro-2-naphtol-4-sulphonate; $C_{20}H_{12}N_3NaO_7S$ = 461.38.

Brownish black powder having a faint, metallic sheen, soluble in alcohol, in *methyl alcohol* and in hot water.

Ether, Diethyl Ether – $(C_2H_5)_2O$ = 74.12.

Analytical reagent grade.

A volatile, highly flammable, colourless liquid, boiling point, about 34°; weight per ml about 0.71g.

Warning –It is dangerous to distil or evaporate ether to dryness unless precautions have been taken to remove peroxides.

Ethyl Acetate –CH_3. $CO_2C_2H_5$ = 88.11.

Analytical reagent grade.

A colourless liquid with a fruity odour; boiling point, about 77°; weight per ml about 0.90g.

Ethyl Alcohol –C_2H_5OH = 46.07.

Absolute Alcohol; Dehydrated Alcohol.

Description –Clear, colourless, mobile, volatile liquid; odour, characteristic and spirituous; taste, burning; hygroscopic. Readily volatilisable even at low temperature and boils at 78° and is flammable.

Solubility –Miscible with water, with solvent ether and with chloroform.

Contains not less than 99.5 per cent w/w or 99.7 per cent v/v of C_2H_5OH..

Identification –Acidity or Alkalinity : Clarity of Solution; Methanol; Foreign organic substances; Isopropyl alcohol and butyl alcohol; Aldehydes and ketones; fusel oil constituents; Non-volatile matter; complies with the requirements described under Alcohol.

Specific gravity –Between 0.7871 and 0.7902, at 25°.

Storage –Store in tightly closed containers in a cool place away from fire and protected from moisture.

Labelling –The label on the container states "Flammable".

Ferric Ammonium Sulphate –Ferric Alum, Fe (NH_4) $(SO_4)_2$, $12H_2O$ = 482.18

Contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $Fe(NH_4)$ $(SO_4)_2$, 12 H_2O.

Description –Pale violet crystals, or a nearly colourless crystalline powder.

Solubility –Soluble in water, yielding a clear yellow or brown solution.

Ferrous iron –Dissolve 1 g in 50 ml of *water*, add 1 ml of *dilute hydrochloric acid* and 1 ml of *potassium ferricyanide solution*; no green or blue colour is produced.

Assay –Weigh accurately about 2 g, dissolve in 10 ml of *dilute hydrochloric acid* and dilute to 50 ml with water, add 3 g of *potassium iodide*, allow to stand for ten minutes titrate the liberated iodine with 0.1 *N sodium thiosulphate*, using starch solution as indicator added towards the end of titrations. Each. ml of 0.1 *N sodium thiosulphate* is equivalent to 0.04822 g of $Fe(NH_4)$ $(SO_4)_2$. $12H_2O$.

Ferric Ammonium Sulphate 0.1N – $FeNH_4(SO_4)_2$, $12H_2O$ = 482.18; 48,22 g in 1000 ml.

Dissolve 50 g of *ferric-ammonium sulphate* in a mixture of 300 ml of water and 6 ml of *sulphuric acid,* dilute with water to 1000 ml, and mix. Standardise the solution as follows :-

Measure accurately about 30 ml of the solution into a glass-stoppered flask, add 5 ml of *hydrochloric acid*, mix, and add a solution of 3 g of *potassium iodide* in 10 ml of *water*. Insert the stopper, allow to stand for ten minutes in the dark, then titrate the liberated iodine with standardised 0.*1N sodium thiosulophate*, adding 3 ml of *starch solution* as the end-point is approached. Perform a blank determination and make any necessary correction. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.04822 g of $FeNH_4(SO_4)_2$, $12H_2O$.

Note – Store 0.1 N Ferric Ammonium Sulphate in tightly-closed, light resistant containers.

Ferric Chloride –Anhydrous Ferric Chloride; $FeCl_3$ = 162.22

Description –Greenish-black crystals or a crystalline powder, free from the orange colour of the hydrated salt, which is readily acquired by exposure to atmospheric moisture.

Solubility –Soluble in *water*, yielding an orange coloured opalescent solution.

Ferrous salts –Dissolve 2.0 g in 100 ml of water, add 2 ml of *phosphoric acid* and titrate with 0.1 *N potassium permanganate* until a pink colour is produced, not more than 0.1 ml is required.

Free chloride –Dissolve 5 g in 10 ml of water and boil the solution; no blue colour is produced on a starch iodide paper exposed to the vapours.

Ferric Chloride Solution –Contains not less than 14.25 per cent and not more than 15.75 per cent w/v of $FeCl_3$.

Description –Clear, Yellowish-brown liquid.

Assay –Dilute 2 ml with 20 ml of water, add 1 ml of *sulphuric acid* and 0.*1 N potassium permanganate* drop by drop until a pink colour persists for five seconds. Add 15 ml of *hydrochloric acid* and 2 g of *potassium iodide*, allow to stand for three minutes, and titrate with 0.1 *N sodium thiosulphate*, using starch solution as indicator added towards the end of titration. Each ml of 0.*1 N sodium thiosulphate* is equivalent to 0.01622 g of $FeCl_3$.

Ferrous Sulphate – $FeSO_4$. $7H_2O$ = 278.0

Description –Transparent, green crystals, or a pale bluish-green, crystalline powder; odourless; taste, metallic and astringent, Efflorescent in dry air. On exposure to moist

air, the crystals rapidly oxidise and become coated with brownish yellow basic ferrous sulphate.

Solubility –Freely soluble in water, very soluble in boiling water, practically insoluble in alcohol.

pH-Between 3.0 and 4.0, determined in a 5.0 per cent w/v solution.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Copper – Dissolve 2 g in 50 ml of *water*, acidify with 1 ml of *dilute sulphuric acid,* saturate with *solution of hydrogen sulphide;* no darkening or precipitate is produced.

Ferrous Sulphate Solution –A 2.0 per cent w/v solution *of ferrous sulphate* in freshly boiled and cooled water.

Ferrous sulphate solution must be freshly prepared.

Ferrous Sulphate Solution, Acid –A 0.45 per cent w/v solution of *ferrous sulphate* in freshly boiled and cooled *water containing* 0.5 ml of hydrochloric acid.

Formaldehyde Solution – Formalin; HCHO =30.03

Formaldehyde Solution is a solution of formaldehyde in water with *methyl alcohol* added to prevent polymerisation. It contains not less than 34.0 per cent w/w and not more than 38.0 per cent w/w of CH_2O.

Description – Colourless liquid; odour, characteristic, pungent and irritating; taste, burning. A slight white cloudy deposit is formed on long standing, especially in the cold, due to the separation of paraformaldehyde. This white deposit disappears on warming the solution.

Solubility – Miscible with *water*, and with *alcohol*.

Acidity – To 10 ml add 10 ml of *carbon dioxide free water* and titrate with 0.1 *N sodium hydroxide* using *bromothymol blue solution* as indicator; not more than 5 ml of 0.1 *N sodium hydroxide* is required.

Wt. per ml – At 20º, 1.079 to 1.094 g.

Assay – Weigh accurately about 3 g and add to a mixture of 50 ml of *hydrogen peroxide solution* and 50 ml of *N sodium hydroxide*, warm on a water-bath until effervescence ceases and titrate the excess of alkali with *N sulphuric acid* using *phenolphthalein solution* as indicator. Repeat the experiment with the same quantities of the same reagents in the same manner omitting the formaldehyde solution. The difference between the titrations represents the sodium hydroxide required to neutralise the formic acid produced by the oxidation of the formaldehyde. Each ml of N sodium hydroxide is equivalent to 0.03003 g of CH_2O.

Storage – Preserve Formaldehyde Solution in well-closed container preferably at a temperature not below 15º

Formaldehyde Solution, Dilute –

Dilute 34 ml of *formaldehyde solution* with sufficient water to produce 100 ml.

Glycerin – $C_3H_8O_3$ = 82.09.

Description – Clear, colourless liquid of syrupy consistency; odourless, taste sweet followed by a sensation of warmth. It is hygroscopic.

Solubility –Miscible with water and with *alcohol*; practically insoluble in chloroform, in solvent ether and in fixed oils.

Acidity –To 50 ml of a 50 per cent w/v solution add 0.2 ml of *dilute phenolphthalein solution*; not more than 0.2 ml of 0.1 *N sodium hydroxide* is required to produce a pink colour.

Wt. per ml –Between 1.252 g and 1.257 g, corresponding to between 98.0 per cent and 100.0 per cent w/w of $C_3H_8O_3$.

Refractive index –Between 1.470 and 1.475 determined at 20º.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Copper –To 10 ml add 30 ml of *water*, and 1 ml of *dilute hydrochloric acid*, and 10 ml of *hydrogen sulphide solution*; no colour is produced.

Iron – 10 g complies with the *limit test* for iron, Appendix 2.3.4.

Heavy metals – Not more than 5 parts per million, determined by Method A on a solution of 4 g in 2 ml of 0.1 *N hydrochloric acid* and sufficient water to produce 25 ml, Appendix 2.3.3.

Sulphate –1 ml complies with the *limit test* for sulphates, Appendix 2.3.7.

Chloride –1 ml complies with the *limit test* for chloride, Appendix 2.3.2.

Acraldehyde and glucose –Heat strongly; it assumes not more than a faint yellow, and not a pink colour. Heat further; it burns with little or no charring and with no odour of burnt sugar.

Aldehydes and related substances – To 12.5 ml of a 50 per cent w/v solution in a glass-stoppered flask add 2.5 ml of *water* and 1 ml of *decolorised magenta solution*. Close the flask and allow to stand for one hour. Any violet colour produced is not more intense than that produced by mixing 1.6 ml of 0.1 *N potassium permanganate* and 250 ml of *water*.

Sugar –Heat 5 g with 1 ml of *dilute sulphuric acid* for five minutes on a water-bath. Add 2 ml of *dilute sodium hydroxide solution* and 1 ml of *copper sulphate solution*. A clear, blue coloured solution is produced. Continue heating on the water-bath for five minutes. The solution remains blue and no precipitate is formed.

Fatty acids and esters – Mix 50 ml with 50 ml of freshly boiled *water* and 50.0 ml of 0.5N *sodium hydroxide,* boil the mixture for five minutes. Cool, add a few drops of *phenolphthalein solution* and titrate the excess alkali with 0.5 *N hydrochloric acid*. Perform a blank determination, not more than 1 ml of 0.5 *N sodium hydroxide* is consumed.

Sulphated ash – Not more than 0.01 per cent, Appendix 2.3.6.

Storage – Store in tightly-closed containers.

Glycerin Solution –Dilute 33 ml of glycerin to 100 ml with water and add a small piece of camphor or liquid phenol.

Hexamine – $(CH_2)_6N_4$ = 140.2

Analytical reagent grade.

Hydrazine Hydrate –NH_2. NH_2. H_2O = 50.06

Analytical reagent grade.

A colourless liquid with an ammonical odour; weight per ml. about 1.03 g.

Hydrochloric Acid –HCl = 36.46

Concentrated Hydrochloric Acid

Description –Clear, colourless, fuming liquid; odour, pungent.

Arsenic –Not more than 1 part per million, Appendix 2.3.1.

Heavy metals –Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner : Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of *dilute acetic acid* to the residue, and add water to make 25 ml, Appendix 2.3.3.

Bromide and iodide –Dilute 5 ml with 10 ml of *water*, add 1 ml of *chloroform*, and add drop by drop, with constant shaking, *chlorinated lime solution*; the chloroform layer does not become brown or violet.

Sulphite –Dilute 1 ml with 10 ml of water, and add 5 drops of *barium chloride solution* and 0.5 ml of 0.001 *N iodine*; the colour of the iodine is not completely discharged.

Sulphate –To 5 ml add 10 mg of sodium bicarbonate and evaporate to dryness on a water bath; the residue, dissolved in *water*; complies with the *limit test for sulphates*, Appendix. 2.3.7.

Free chlorine –Dilute 5 ml with 10 ml of freshly boiled and cooled *water*, add 1 ml of cadmium *iodide solution*, and shake with 1 ml of *chloroform*; the chloroform layer does not become violet within one minute.

Sulphated ash –Not more than 0.01 per cent, Appendix 2.3.6.

Assay –Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrare with *N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.03646 g of HCl.

Storage –Store in glass-stoppered containers at a temperature not exceeding 30º.

Hydrochloric Acid, x N –Solution of any normality x N may be prepared by diluting 84 x ml of *hydrochloric acid* to 1000 ml with *water*.

Hydrochloric Acid –(1 per cent w/v) Dilute 1 g of hydrochloric *acid* to 100 ml with *water.*

Dilute Hydrochloric Acid –

Description –Colourless liquid.

Arsenic, heavy metals bromoide and iodide, sulphate, free chlorine –Complies with the tests described under Hydrochloric Acid, when three times the quantity is taken for each test.

Assay –Weigh accurately about 10 g and carry out the Assay described under Hydrochloric Acid.

Storage –Store in stoppered containers of glass or other inert material, at temperature below 30º.

Hydrochloric Acid, N – HCl = 36.460

36.46 g in 1000 ml

Dilute 85 ml of hydrochloric acid with water to 1000 ml and standardise the solution as follows :

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270º for one hour. Dissolve it in 100 ml of *water* and add two drops of *methyl red solution*. Add the acid slowly from a burette with constant stirring, until the solution becomes faintly pink. Heat again to boiling and titrate further as necessary until the faint pink colour no longer affected by continued boiling. Each 0.5299 g of *anhydrous* sodium carbonate is equivalent to 1 ml of N hydrochloric acid.

Hydrochloric Acid, Iron-Free –Hydrochloric acid, which complies with the following additonal test. Evaporate 5 ml on a water-bath nearly to dryness, add 40 ml of water, 2 ml of a 20 per cent w/v solution of citric acid and two drops of thioglycollic acid, mix, make alkaline with *dilute ammonia solution*, and dilute to 50 ml with water; no pink colour is produced.

Hydrogen Peroxide Solution – (20 Vol.) H_2O_2 = 34.02

Analytical reagent grade of commerce or *hydrogen peroxide solution* (100 Vol.) diluted with 4 volumes of water.

A colourless liquid containing about 6 per cent w/v of H_2O_2; weight per ml, about 1.02 g.

Hydrogen Sulphide – H_2S =34.08

Use laboratory cylinder grade, or prepare the gas by action of hydrochloric acid, diluted with an equal volume of *water*, on iron sulphide, the resulting gas is washed by passing it through water.

A colourless, poisonous gas, with a characteristic unpleasant odour.

Hydrogen Sulphide Solution –A recently prepared, saturated solution of hydrogen sulphide in water at 20º.

Hydrogen Sulphide solution contains about 0.45 per cent w/v of H_2S.

Hydroxylamine Hydrochloride; Hydroxylammonium Chloride – NH_2OH, HCl = 69.49

Contains not less than 97.0 per cent w/w of NH_2OH, HCI

Description – Colourless crystals, or a white, crystalline powder.

Solubility – Very soluble in water; soluble in alcohol.

Free acid – Dissolve 1.0 g in 50 ml of *alcohol*, add 3 drops of *dimethyl yellow solution* and titrate to the full yellow colour with *N sodium hydroxide*; not more than 0.5 ml of *N sodium hydroxide* is required.

Sulphated ash – Not more than 0.2 per cent, Apendix 2.3.6.

Assay – Weigh accurately about 0.1 g and dissolve in 20 ml of water, add 5 g of ferric ammonium sulphate dissolve in 20 ml of water, and 15 ml of *dilute sulphuric acid*, boil for five minutes, dilute with 200 ml of water, and titrate with 0.1 *N potassium permanganate*. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.003475 g of NH_2OH, HCl.

Hydroxylamine Hydrochloride Solution – Dissolve 1 g of *hydroxylamine hydrochloride* in 50 ml of *water* and add 50 ml of *alcohol,* 1 ml of *bromophenol blue solution* and 0.1 *N sodium hydroxide* until the solution becomes green.

***Indigo Carmine** – $C_{16}H_8N_2Na_2O_8S_2$ =466.4

Analytical reagent grade.

A deep blue powder, or blue granules with a coppery lustre.

Indigo Carmine Solution – To a mixture of 10 ml of *hydrochloric acid* and 990 ml of a 20 per cent w/v solution of sulphuric acid in water, add sufficient indigo carmine to produce a solution, which complies with the following test.

Add 10 ml to a solution of 1.0 mg of potassium nitrate in 10 ml of *water*, add, rapidly, 20 ml of sulphuric acid and heat to boiling; the blue colour is just discharged in one minute.

Iodine – I2 = 253.8

Description – Heavy, bluish-black, brittle, rhombic prisms or plates with a metallic lustre; odour characteristic; volatile at ordinary temperatures.

Solubility – Very slightly soluble in *water*; soluble in *alcohol,* freely soluble in carbon disulphide and in *chloroform,* in *solvent ether*, *in carbon tetrachloride* and in concentrated aqueous solutions of iodides.

Chloride and Bromide – Triturate 3.5 g thoroughly with 35 ml of *water*, filter and decolorise the filtrate by the addition of a little *zinc powder*. To 25 ml of the filtrate so obtained, add 5 ml of d*ilute ammonia solution,* and then 5 ml of *silver nitrate solution* added gradually, filter; dilute the filtrate to 50 ml, and acidify gradually with 4 ml of nitric acid; the opalescence in the *limit test* for chloride, Appendix 2.3.1.

Cyanides – To 5 ml of the filtrate obtained in the test for *chloride* and *bromide* add a few drops of *ferrous sulphate solution* and 1 ml of *sodium hydroxide solution*, warm gently and acidify with hydrochloric acid, no blue or green colour is produced.

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*Indian ink –General purpose grade.

Non-volatile matter – Leaves not more than 0.1 per cent as residue when volatilised on a water-bath.

Assay – Weigh accurately about 0.5 g and dissolve in a solution of 1 g of *potassium iodide* in 5 ml of *water*. Dilute to 250 ml with *water*, add 1 ml of *dilute acetic acid*, and *titrate* with 0.1 *N sodium thiosulphate, using starch solution* as indicator. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.01269 g of I.

Storage – Store in glass-stoppered bottles or in glass or earthen-ware containers with well waxed bungs.

Iodine, 0.1N – I = 126.90; 12.69 g in 1000 ml.

Dissolve about 14 g of *iodine* in a solution of 36 g of *potassium iodide* in 100 ml of water, add three drops of *hydrochloric* acid, dilute with water to 100 ml and standardise the solution as follows :

Weigh accurately about 0.15 g of *arsenic trioxide*, previously dried at 105° for one hour, and dissolve in 20 ml of *N Sodium hydroxide* by warming, if necessary. Dilute with 40 ml of water, add two drops of *methyl orange solution* and follow with *dilute hydrochloric acid* until the yellow colour is changed to pink. Then add 2 g of *sodium bicarbonate*, dilute with 50 ml of water, and add 3 ml of starch solution, slowly add the iodine solution from a burette until a permanent blue colour is produced. Each 0.004946 g of arsenic trioxide is equivalent to 1 ml of 0.1*N iodine*.

Iodine Solution. – Dissolve 2.0 g of iodine and 3 g of *potassium iodide* in water to produce 100 ml.

Kieselguhr – A natural diatomaceous earth, purified by heating with dilute hydrochloric acid, washing with water and drying.

Lactic Acid – $CH_3CH(OH).COOH$ = 90.08

Analytical reagent grade of commerce

Lactophenol – Dissolve 20 g of *phenol* in a mixture of 20 g of *lactic acid*, 40 g of *glycerol*, and 20 ml of water.

Lead Acetate – Sugar of lead; $(CH_3CO_2)_2$ Pb, $3H_2O$ = 379.33

Contains not less than 99.5 per cent and not more than the equivalent of 104.5 per cent of C4H6O4Pb, $3H_2O$.

Description –Small, white, transparent, monoclinic prisms, or heavy, crystalline masses; odour, acetous, taste, sweet and astringent. Efflorescent in warm air. Becomes basic when heated.

Solubility –Freely soluble in *water*, and in *glycerin*; sparingly soluble in *alcohol*.

Water-insoluble matter –Dissolve 1 g in 10 ml of recently boiled and cooled *water; a* solution is produced which is, at most, faintly opalescent and becomes clear on the addition of one drop of *acetic acid*.

Chloride –1 g complies with the *limit test* for chlorides, Appendix 2.3.1.

Copper, iron, silver, and zinc – Dissolve 0.5 g in 10 ml of *water*, add 2 ml of dilute *sulphuric acid*, allow to stand for thirty minutes, and filter; to the filtrate add an excess of *potassium ferrocyanide solution;* no precipitate or colour is produced.

Assay –Weigh accurately about 0.8 g and dissolve in a mixture of 100 ml of *water* and 2 ml of *acetic acid*, add 5 g of hexamine, titrate with 0.05 *M disodium ethylenediaminetetraacetate*, using 0.*2 ml of xylenol orange solution* as indicator, until the solution becomes pale bright yellow. Each ml of 0.05 *M disodium ethylenediaminetetraacetate* is equivelent to 0.01897 g of $C_4H_6O_4Pb$, $3H_2O$.

Storage –Preserve Lead Acetate in a well-closed container.

Lead Acetate Solution –A 10.0 per cent w/v solution *of lead acetate in carbon dioxide-free water*.

Lead Nitrate – $Pb(NO_3)_2$ = 331.21

Contains not less than 99.0 per cent of $Pb(NO_3)_2$

Description –Colourless or white crystals, or a white crystalline powder.

Solubility –Soluble in water, forming a clear, colourless solution.

Assay –Weigh accurately about 0.3 g and dissolve in 150 ml of water. Add 5 ml of dilute *acetic acid*, heat to boiling, add a slight excess of *potassium chromate solution*, and boil gently until the precipitate becomes granular; collect the precipitate in a Gooch crucible, wash it with hot water, and dry to constant weight at 120º. Each g of residue is equivalent to 1.025 g of $Pb(NO_3)_2$.

Lead Solution, Standard –See limit test for heavy metals, Appendix 2.3.3.

Liquid Paraffin –General reagent grade.

Liquid paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

A transparent, colourless, oily liquid, free or nearly free from fluorescence by day light; odourless and tasteless when cold, and develops not more than a faint odour of petroleum when heated.

Solubility –Practically insoluble in water, and in alcohol; soluble in chloroform, in solvent ether and in volatile oils.

Wt. per ml. –At 25º, 0.860 to 0.904 g.

Litmus –Fragments of blue pigment prepared from various species of *Rocella lecanora* or other *lichens*. It has a characteristic odour.

Partly soluble in water and in alcohol. Gives a red colour with acids and a blue colour with alkalies (pH range, 5.0 to 8.0).

Litmus Solution – Boil 25 g of coarsely powdered litmus with 100 ml of *alcohol (90 per cent)* under a reflux condenser for one hour, and pour away the clear liquid; repeat this operation using two successive quantities, each of 75 ml, of *alcohol (90 per cent)*. Digest the extracted litmus with 250 ml of water.

Litmus Paper, Blue – Boil 10 parts of coaresely powdered litmus under reflux for one hour with 100 parts of alcohol, decant the alcohol and discard. Add to the residue a mixture of 45 parts of alcohol and 55 parts of water. After two days decant the clear

liquid. Impregnate the strips of filter paper with the extract and allow to dry the paper; complies with the following test –

Sensitivity – Immerse a strip measuring 10 mm x 60 mm in 100 ml of a mixture of 10 ml of 0.02 *N hydrochloric acid* and 90 ml of *water*. On shaking the paper turns red within forty five seconds.

Litmus Paper, Red – To the extract obtained in the preparation of blue litmus paper add 2 *N hydrochloric acid* drop-wise until the blue colour becomes red. Impregnate strips of filter paper with the solution and allow to dry. The paper complies with the following test :

Sensitivity – Immerse a strip measuring 10 mm x 60 mm in 100 ml of 0.002 *N sodium hydroxide*. On shaking the paper turns blue within forty-five minutes.

Magenta Basic – Fuchsin; Rosaniline hydro-chloride; $[(H_2N.\ C_6H_4)_2C : C_6H_3(CH_3) : NH_2{+}]Cl^-$ = 337.85.

The hydrochloride of rosaniline of such a purity that when used in the preparation of decolourised solution of magenta, a nearly colourless solution is obtained.

Description –Dark red powder, or green crystals with a metallic lustre.

Solubility –Soluble in water, giving a deep reddish-purple solution.

Sulphated ash –Not more than 5.0 per cent, Appendix 2.3.6.

Magenta Solution, Decolorised –Dissolve 1 g of basic *magenta* in 600 ml of water and cool in an ice bath; add 20 g of *sodium sulphite* dissolved in 100 ml of water; cool in an ice-bath and add, slowly with constant stirring, 10 ml of hydrochloric acid; dilute with water to 1000 ml.

If the resulting solution is turbid, it should be filtered and if brown in colour, it should be shaken with sufficient decolourising charcoal (0.2 to 0.3 g) to render it colourless and then filtered immediately. Occasionally it is necessary to add 2 to 3 ml *of hydrochloric acid*, followed by shaking, to remove the little residual pink colour. The solution resulting from any of the foregoing modifications should be allowed to stand over-night before use.

Decolourised magenta solution should be protected from light.

Magnesium Carbonate –Light hydrated basic grade of commerce, containing 42 to 45 per cent of MgO and complying with the following test :

Ammonia –Dissolve 0.50 g in 4 ml of 2 M hydrochloric acid, boil to remove carbon dioxide, and dilute with *water* to 95 ml. Add 5 ml of 5 *M sodium hydroxide* and allow to stand for one hour. Dilute 40 ml of the clear liquid to 50 ml with water and add 2 ml of *alkaline potassium-mercuric iodide solution*. Any yellow colour produced is not deeper than that produced by adding 2 ml of *alkaline potassium mercuric iodide solution* to a mixture of 44 ml of water, 2 ml of *ammonium chloride solution*, 2 ml of 2 *M hydrochloric acid* and 2 ml of 5 *M sodium hydroxide*.

Magnesium Sulphate – $MgSO_4$, $7H_2O$ = 246.47

Description –Colourless, crystals, usually needle-like; odourless, taste, cool, saline and bitter. Effloresces in warm dry air.

Solubility –Freely soluble in water; sparingly soluble in alcohol. Dissolves slowly in glycerin.

Acidity or alkalinity – 1 g dissolved in 10 ml of water is neutral to *litmus solution*.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Iron –2 g dissolved in 20 ml of water complies with the limit test for iron, Appendix 2.3.4.

Heavy metals –Not more than 10 parts per million, determined by Method A on a solution prepared by dissolving 2.0 g in 10 ml of water, 2.0 ml of *of dilute acetic acid* and sufficient *water* to make 25 ml, Appendix 2.3.3.

Zinc –Dissolve 2 g in 20 ml of water and acidify with 1 ml of *acetic acid*. No turbidity is produced immediately on the addition of few drops of *potassium ferrocyanide solution*.

Chloride –1 g complies with the *limit test for chlorides*, Appendix 2.3.2.

Loss on ignition –Between 48.0 per cent and 52.0 per cent, determined on 1.0 g by drying in an oven at 105º for two hours and igniting to constant weight at 400º.

Assay –Weigh accurately about 0.3 g and dissolve in 50 ml of *water*. Add 10 ml of *strong ammonia-ammonium chloride solution*, and titrate with 0.05 *M disodium ethylenediaminetetraacetate* using 0.1 g of *mordant black* II mixture as indicator, until the pink colour is discharged from the blue. Each ml of 0.05 *M disodium ethylenediaminetetraacetate* is equivalent to 0.00602 g of $MgSO_4$.

Storage –Store in well-closed containers.

Magnesium Sulphate, Dried, – $MgSO_4$

Dried, general reagent grade of commerce.

Magnesium Sulphate Solution, Ammoniacal –Dissolve 10 g of *magnesium sulphate* and 20 g of *ammonium chloride* in 80 ml of *water*, and add 42 ml of *5 M ammonia*. Allow to stand for a few days in a well closed container; decant and filter.

Mercuric Chloride –$HgCl_2$ =271.50.

Contains not less than 99.5 per cent of HgCl2;

Description –Heavy, colourless or white, crystalline masses, or a white crystalline powder.

Solubility –Soluble in *water*; freely soluble in *alcohol*.

Non-volatile matter –When volatilised, leaves not more than 0.1 per cent of residue.

Assay – Weigh accurately about 0.3 g and dissolve in 85 ml of *water* in a stoppered-flask, add 10 ml of *calcium chloride solution*, 10 ml of *potassium iodide solution*, 3 ml of *formaldehyde solution and* 15 ml of *sodium hydroxide solution*, and shake continuously for two minutes. Add 20 ml of acetic acid and 35 ml of 0.1 *N iodine*. Shake continuously for about ten minutes, or until the precipitated mercury is completly

redissolved, and titrate the excess of iodine with 0.1 *N sodium thiosulphate*. Each ml of 0.1 *N iodine is* equivalent to 0.01357 g of $HgCl_2$.

Mercuric Chloride, 0.2 M – Dissolve 54.30 g of *mercuric chloride* in sufficient water to produce 1000 ml.

Mercuric Chloride Solution – A 5.0 per cent w/v solution of *mercuric chloride* in water.

Mercuric Oxide, Yellow – HgO = 216.59.

Contains not less than 99.0 per cent of HgO, calculated with reference to the substance dried at 105º for one hour.

Description – Orange-yellow, heavy, amorphous powder; odourless, stable in air but becomes discoloured on exposure to light.

Solubility –Practically insoluble in water and in *alcohol*; freely soluble in *dilute hydrochloric acid* and in dilute *nitric acid*, forming colourless solutions.

Acidity or alkalinity –Shake 1 g with 5 ml of *water* and allow to settle; the supernatant liquid is neutral to *litmus solution*.

Mercurous salts –A solution of 0.5 g in 25 ml of *dilute hydrochloric acid* is not more than slightly turbid.

Chloride – To 0.2 g add 1 g of zinc powder and 10 ml of *water*. Shake occasionally during ten minutes and filter; the solution complies with the *limit test* for chlorides, Appendix 2.3.2.

Sulphated ash –When moistened with *sulphuric acid* in a silica dish and heated strongly to constant weight, leaves not more than 0.5 per cent of residue.

Assay –Weigh accurately about 0.4 g, dissolve in 5 ml of nitric acid and 10 ml of *water* and *dilute* with *water* to 150 ml. Titrate with 0.1 *N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Carry out the titration at a temperature not above 20º. Each ml of 0.*1 N ammonium thiocyanate* is equivalent to 0.01083 g of HgO.

Storage –Preserve Yellow Mercuric Oxide in a well-closed container, protected from light.

Mercuric Potassium Iodide Solution – See Potassium - Mercuric Iodide solution.

Mercuric Sulphate –Mercury (II) Sulphate $HgSO_4$= 296.68

Contains not less than 99.0 per cent of $HgSO_4$

Description- A white; crystalline powder, hydrolyses in water.

Solubility – Soluble in *dilute sulphuric acid.*

Chloride –Dissolve 2.0 g in a mixture of 2 ml of *dilute sulphuric acid* and 10 ml of *water*. Add 2 g of zinc powder, shake frequently for five minutes and filter. The filtrate complies with the *limit test* for *chlorides*, Appendix 2.3.2.

Nitrate –Dissolve 0.40 g in a mixture of 9 ml of water and 1 ml of dilute sulphuric acid, add 1 ml of indigo carmine solution and 10 ml of *nitrogen-free sulphuric acid* and heat to boiling, the blue colour is not entirely discharged.

Assay –Dissolve 0.6 g in a mixture of 10 ml of *dilute nitric acid* and 40 ml of *water*. Titrate with 0.1 *N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of 0.1 *N ammonium thiocyanate* is equivalent to 0.01483 g of $HgSO_4$.

Mercury Sulphate Solution – Mix 5 g of *yellow mercuric oxide* with 40 ml of *water*, and while stirring add 20 ml of *sulphuric acid*, and 40 ml of *water*, and stir until completely dissolved.

Methyl Alcohol : Methanol : CH_3OH = 32.04.

Description –Clear, Colourless liquid with a characteristic odour.

Solubility –Miscible with water, forming a clear colourless liquid.

Specific Gravity – At 25º, not more than 0.791.

Distillation range – Not less than 95 per cent distils between 64.5º and 65.5º.

Refractive Index –At 20º, 1.328 to 1.329.

Acetcone –Place 1 ml in a *Nessler cylinder*, add 19 ml of water, 2 ml of a 1 per cent w/v solution of *2-nitrobenzaldehyde* in *alcohol (50 per cent)*, 1 ml of 30 per cent w/v *solution of sodium hydroxide* and allow to stand in the dark for fifteen minutes. The colour developed does not exceed that produced by mixing 1 ml of standard acetone solution, 19 ml of water, 2 ml of the solution of *2-nitrobenzaldehyde* and 1 ml of the *solution of sodium hydroxide* and allowing to stand in the dark for fifteen minutes.

Acidity –To 5 ml add 5 ml of *carbon dioxide-free water*, and titrate with 0.1 *N sodium hydroxide*, using *bromothymol blue solution* as indicator; not more than 0.1 ml is required.

Non-volatile matter – When evaporated on a water-bath and dried to constant weight at 105º, leaves not more than 0.005 per ceant w/v of residue.

Methyl Alcohol, Dehydrated –Methyl alcohol, which complies with the following additional requirement.

Water –Not more than 0.1 per cent w/w.

Methylene Blue –$C_{16}H_{18}ClN_3S$, $3H_2O$. Tetramethylthionine chloride.

A dark green or bronze crystalline powder, freely soluble in water, soluble in alcohol.

Loss on drying –Not less than 18 per cent and not more than 22 per cent, determined by drying in an oven at 100º to 105º.

Methylene Blue Solution – Dissolve 0.18 g of *methylene blue* in 100 ml of *water*. To 75 ml of this solution, add 5 ml of 0.1 *N sodium hydroxide* and 20 ml of water.

Methyl Orange – Sodium-p-dimethyla-mineazobenzene sulphate, $C_{14}H_{14}O_3N_3SNa$.

An orange-yellow powder or crystalline scales, slightly soluble in cold water; insoluble in alcohol; readily soluble in hot water.

Methyl Orange Solution –Dissolve 0.1 g of methyl orange in 80 ml of water and dilute to 100 ml with alcohol.

Test for sensitivity –A mixture of 0.1 ml of the methyl orange solution and 100 ml freshly boiled and cooled water is yellow. Not more than 0.1 ml of 0.1 N hydrochloric acid is required to change the colour to red.

Colour change – pH 3.0 (red) to pH 4.4 (yellow).

Methyl Red –p-Dimethylaminoazobenzene-o-carboxylic acid, $C_{15}H_{15}O_2N_3$.

A dark red powder or violet crystals, sparingly soluble in *water*; soluble in alcohol.

Methyl red solution – Dissolve 100 mg in 1.86 ml of 0.1 *N sodium hydroxide* and 50 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity – A mixture of 0.1 ml of the *methyl red solution* and 100 ml of freshly boiled and cooled *water* to which 0.05 ml of 0.02 *N hydrochloric acid* has been added is red. Not more than 0.01 ml of 0.02 *N sodium hydroxide* is required to change the colour to yellow.

Colour change – pH 4.4 (red) to pH 6.0 (yellow).

Molish's Reagent –Prepare two solutions in separate bottles, with ground glass stoppers :

(a) Dissolve 2 g of ??naphthol in 95 per cent alcohol and make upto 10 ml with alcohol (??naphthol can be replaced by thymol or resorcinol). Store in a place protected from light. The solution can be used for only a short period.

(b) Concentrated sulphuric acid.

Mordant Black II –See Eriochrome black T.

Mordant Black II Mixture –*Mordant black mixture.*

A mixture of 0.2 part of Mordant Black II with 100 parts of sodium chloride. Mordant Black II Mixture should be recently prepared.

?Naphthol – 1-Naphthol; $C_{10}H_7OH$=144.17.

Description – Colourless or white crystals or a white, crystalline powder; odour, characteristic.

Solubility –Freely soluble in alcohol yielding not more than slightly opalescent, colourless or almost colourless solution, with no pink tint.

Melting range –93° to 96°.

Sulphated ash –Not more than 0.05 per cent, Appendix 2.3.6.

?Naphthol Solution – 1-Naphthol solution.

Dissolve 1 g of *?naphthol* in a solution of 6 g of *sodium hydroxide* and 16 g of *anhydrous sodium carbonate* in 100 ml of water.

??naphthol solution must be prepared immediately before use.

1-Naphthylamine –$C_{10}H_9N$ = 143.2 – Analytical reagent grade.

Almost colourless crystals, or a white crystalline powder; melting point, about 50º.

Naphthylamine-Sulphanilic Acid Reagent –Immediately before use mix equal volumes of solutions A and B prepared as follows :

Solution A –Dissolve 0.5 g of sulphuric acid in 30 ml of 6 M *acetic acid* and dilute to 150 ml with water.

Solution B –Dissolve 0.15 g of 1 naphthylamine in 30 ml of 6 M *acetic acid and* dilute to 150 ml with water.

Ninhydrin Reagent – 30 mg ninhydrin is dissolved in 10 ml n-butanol, followed by 0.3 ml of 98 % acetic acid.

Nitric Acid –Contains 70.0 per cent w/w of HNO3 (limits, 69.0 to 71.0). About 16 N in strength.

Description –Clear, colourless, fuming liquid.

Wt. per ml. – At 20º, 1.41 to 1.42 g.

Copper and Zinc –Dilute 1 ml with 20 ml of water, and add a slight excess of dilute ammonia solution; the mixture does not become blue. Pass hydrogen sulphide; a precipitate is not produced.

Iron –0.5 ml of complies with the limit test for iron, Appendix 2.3.4.

Lead –Not more than 2 parts per million, Appendix 2.3.5.

Chloride –5 ml neutralised with dilute ammonia solution, complies with the limit test for chlorides, Appendix 2.3.2.

Sulphates –To 2.5 ml add 10 mg of sodium bicarbonate and evaporate to dryness on a water-bath, the residue dissolved in water, complies with the limit test for sulphates, Appendix 2.3.7.

Sulphated ash – Not more than 0.01 per cent w/w, Appendix 2.3.6.

Assay – Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrate with N Sodium hydroxide, using methyl orange solution as indicator. Each ml of N sodium hydroxide is equivalent to 0.06301 g of HNO_3.

Nitric Acid, XN –Solutions of any normality XN may be prepared by diluting 63x ml of nitric acid to 1000 ml with water.

Nitric Acid, Dilute –Contains approximately 10 per cent w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

2-Nitrobenzaldehyde –0-Nitrobenzaldehyde $NO_2C_6H_4CHO$ =151.12.

Description –Yellow needles, odour, resembling that of benzaldehyde.

Solubility –Soluble in alcohol.

Melting range –40º to 45º.

Sulphated ash – Not more than 0.1 per cent, Appendix 2.3.6.

Oxalic Acid – $(CO_2H)_2$, $2H_2O$ =126.07.

Contains not less than 99.0 per cent of $C_2H_2O_4$, $2H_2O$, as determined by the methods A and B under the Assay.

Description –Colourless crystals.

Solubility – Soluble in water and in alcohol.

Chloride – To 1 g dissolved in 20 ml of water add 5 ml. of dilute *nitric acid* and 1 drop of silver nitrate solution; no turbidity is produced.

Sulphated ash –Not more than 0.05 per cent, Appendix 2.3.6.

Assay –

Weigh accurately about 3 g and dissolve in 50 ml of carbon dioxide free water and titrate with N sodium hydroxide, using phenolphtahalein solution as indicator. Each ml of N sodium hydroxide is equivalent to 0.06304 of $C_2H_2O_4$, $2H_2O$.

Weigh accurately about 3 g, dissolve in water, and add sufficient water to produce 250 ml. To 25 ml of this solution add 5ml of sulphuric acid previously diluted with a little water, and titrate at a temperature of about 70º with 0.1N potassium permanganate. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.006303 g of $C_2H_2O_4$, $2H_2O$.

Oxalic Acid, 0.1 N – $C_2H_2O_4$, $2H_2O$ = 126.07, 6.303 g in 1000 ml.

Dissolve 6.45 g of oxalic acid in sufficient water to produce 1000 ml and standardise the solution as follows:

Pipette 30 ml of the solution into a beaker, add 150 ml of water, 7 ml of *sulphuric acid* and heat to about 70º. Add slowly from a burette freshly standardised 0.1 *N potassium permanganate* with constant stirring, until a pale-pink colour, which persists for fifteen seconds, is produced. The temperature at the conclusion of the titration should not be less than 60°. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.006303 g of $H_2C_2O_4$, $2H_2O$.

Petroleum Light – Petroleum Spirit

Description –Colourless, very volatile, highly flammable liquid obtained from petroleum, consisting of a mixture of the lower members of the paraffin series of hydrocarbons and complying with one or other of the following definitions :

Light Petroleum –(Boiling range, 30º to 40º).

Wt. per ml. –At 20º, 0.620 to 0.630 g.

Light Petroleum –(Boiling range, 40º to 60º).

Wt. per ml –At 20º, 0.630 to 0.650 g.

Light Petroleum –(Boiling range, 60º to 80º).

Wt. per ml. –At 20°, 0.670 to 0.690.

Light Petroleum –(Boiling range, 80° to 100°).

Wt. per ml. –At 20°, 0.700 to 0.720

Light Petroleum –(Boiling range, 100° to 120°).

Wt. per ml –At 20°, 0.720 to 0.740 g.

Light Petroleum –(Boiling range, 120° to 160°).

Wt. per ml –At 20°, about 0.75 g.

Non-volatile matter – When evaporated on a water-bath and dried at 105°, leaves not more than 0.002 per cent w/v of residue.

Phenacetin – $C_{10}H_{13}O_2N$ = 179.2

Analytical reagent grade.

White, glistening, crystalline scales, or a fine, white, crystalline powder; odourless; taste, slightly bitter.

Melting range –134° to 136°.

Phenol – C_6H_5OH = 94.11

Analytical reagent grade.

Caustic, deliquescent crystals with a characteristic odour; freezing point, about 41°.

Phenol Liquified –General reagent grade.

A solution in water containing about 80 per cent w/w C_6H_6O.

Phenol Red –$C_{19}H_{14}O_5S$. Phenolsulphonphthalein.

A light to dark red crystalline powder, very slightly soluble in water, slightly soluble in alcohol, soluble in dilute alkaline solutions.

Phenol Red Solution –Dissolve 0.10 g of *phenol red* in 2.82 ml of 0.1 *N sodium hydroxide*, and add 20 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –A mixture of 0.1 ml of the *phenol red solution* in 100 ml of freshly boiled and cooled water is yellow. Not more than 0.1 of 0.02 *N* sodium hydroxide is required to change the colour to red-violet.

Colour change - pH 6.8 (yellow) to pH 8.4 (red-violet).

Phenolphthalein –$C_{20}H_{14}O_4$.

A white to yellowish-white powder, practically insoluble in water, soluble in alcohol.

Phenolphthalein Solution –Dissolve 0.10 g in 80 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –To 0.1 ml of the *phenolphthalein solution* add 100 ml of freshly boiled and cooled water, the solution is colourless. Not more than 0.2 ml of 0.02 *N sodium hydroxide* is required to change the colour to pink.

Colour change –pH 8.2 (colourless) to pH 10.0 (red)

Phloroglucinol – 1 : 3 : 5 – Trihydroxybenzene , $C_6H_3(OH)_3$, $2H_2O$.

Description – White or yellowish crystals or a crystalline powder.

Solubility –Slightly soluble in water; soluble in *alcohol*, and in *solvent ether*.

Melting range –After drying at 110° for one hour, 215° to 219°.

Sulphated ash – Not more than 0.1 per cent, Appendix 2.3.6.

Phloroglucinol should be kept protected from light.

Phloroglucinol Solution – A 1.0 per cent w/v solution of phloroglucinol in alcohol (90 per cent).

Phosphoric Acid – H_3PO_4 = 98.00.

(Orthophosphoric Acid; Concentrated Phosphoric Acid).

Description –Clear and colourless syrupy liquid, corrosive.

Solubility –Miscible with water and with alcohol.

Hypophoshorous and phosphorous acid – To 0.5 ml add 10 ml of water and 2 ml of *silver nitrate solution* and heat on a waterbath for five minutes; the solution shows no change in appearance.

Alkali phosphates - To 1 ml in a graduated cylinder add 6 ml of *solvent ether* and 2 ml of *alcohol*; no turbidity is produced.

Chloride –1 ml complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –0.5 ml complies with the limit test for sulphate, Appendix 2.3.7.

Arsenic – Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 10 parts per million, determined by Method A on a solution prepared by diluting 1.2 ml with 10 ml of *water*, neutralising with dilute *ammonia solution*, adding sufficient dilute *acetic acid* to render the solution acidic and finally diluting to 25 ml with *water*, Appendix 2.3.3.

Iron –0.1 ml complies with the limit test for iron, Appendix 2.3.4.

Aluminium and calcium –To 1 ml add 10 ml of water and 8 ml of dilute *ammonia solution* the solution remains clear.

Assay –Weigh accurately about 1 g. and mix with a solution of 10 g of *sodium chloride* in 30 ml of water. Titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.049 g of H_3O_4

Storage –Store in a well-closed glass containers.

Phosphoric Acid, xN –

Solutions of any normality, x N may be prepared by diluting 49 x g of *phosphoric acid* with water to 1000 ml.

Phosphoric Acid, Dilute –

Contains approximately 10 per cent w/v of H_3O_4.

Dilute 69 ml of *phosphoric acid* to 1000 ml with water.

Piperazine Hydrate –$C_4H_{10}N_2$, $6H_2O$ = 194.2.

General reagent grade of commerce.

Colourless, glossy, deliquescent crystals, melting point, about 44º.

Potassium Antimonate –$KSbO_3$, $3H_2O$ =262.90.

Contains not less than 40.0 per cent of Sb.

Description – White, crystalline powder.

Solubility –Sparingly soluble in water, very slowly soluble in cold, but rapidly soluble on boiling.

Assay –Weigh accurately about 0.3 g, and dissolve in 100 ml of water, add 2 ml of dilute *hydrochloric acid*, and pass in *hydrogen sulphide* until the *antimony* is completely precipitated. Add 2 ml of *hydrochloric acid* and again pass in *hydrogen sulphide.* Boil, filter, wash the precipitate with hot *water* saturated with *hydrogen sulphide,* and dissolve the precipitate in 25 ml of *hydrochloric acid*. Boil to remove *hydrogen sulphide*, and dilute to 50 ml with *water.* Add 2 g of *sodium potassium tartrate,* neutralise carefully with *sodium carbonate,* add 2 g *sodium bicarbonate,* and titrate with 0.1 *N iodine, using starch solution* as indicator. Each ml of 0.1 *N iodine* is equivalent to 0.006088 g of Sb.

Potassium Antimonate Solution –Boil 2 g of *potassium antimonate* with 95 ml of *water* until dissolved. Cool rapidly and add 50 ml of *potassium hydroxide solution* and 5 ml of *N sodium hydroxide.* Allow to stand twenty-four hours, filter and and sufficient water to produce 150 ml.

Sensitivity to sodium –To 10 ml add 7 ml of 0.1 M *sodium chloride,* a white crystalline precipitate is formed within fifteen minutes.

Potassium Antimonate Solution should be freshly prepared.

Potassium Bisulphate – Potassium Hydrogen Sulphate; $KHSO_4$ = 136.16.

Contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $KHSO_4$.

Description – Fused, white lumps; hygroscopic.

Solubility –Very soluble in water, giving an acid solution.

Iron–2 g complies with the limit test for iron, Appendix 2.3.4.

Assay– Weigh accurately about 4.5 g, dissolve in 50 ml of *water* and titrate with *N sodium hydroxide* using *methyl red solution as* indicator. Each ml of *N sodium hydroxide* is equivalent to 0.1362 g of $KHSO_4$

Potassium Bromate – $KBrO_3$ = 167.00

Contains not less than 99.8 per cent of $KBrO_3$ calculated with reference to the substance dried to constant weight at 105º.

Description –White, crystalline powder.

Solubility – Soluble in *water,* freely soluble in boiling water, almost insoluble in *alcohol.*

Acidity or Alkalinity – A 5 per cent w/v solution in *water* is clear and colourless and neutral to *litmus solution.*

Sodium – A warm 10 per cent w/v solution in *water,* tested on platinum wire, imparts no distinct yellow colour to a colourless flame.

Bromide – To 20 ml of a 5 per cent w/v solution in *water,* add 1 ml of 0.1 *N sulphuric acid;* no yellow colour develops within one minute, comparison being made with a control solution to which no acid has been added.

Sulphate – 1 g complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay – Weigh accurately about 1 g, dissolve in water and dilute to 250 ml. To 25 ml of this solution add 3 g of *potassium iodide* and 10 ml of *hydrochloric acid,* dilute with 100 ml of water and titrate with 0.1 *N sodium thiosulphate,* using *starch solution* as indicator. Each ml of 0.1 *N sodium thiosulphate* is equivalent 0.002783 g of $KBrO_3$.

Potassium Bromide – KBr = 119.0

Analytical reagent grade.

Potassium Bromide, 0.001 N –

Dissolve 0.1190 g of *potassium bromide* in sufficient *water* to produce 1000 ml.

Potassium Carbonate –$K2CO_3$ = 138.21

Contains not less than 98.0 per cent of $K2CO_3$.

Description –White, granular powder, hygroscopic.

Solubility –Very soluble in *water,* forming a clear solution.

Iron – 1 g, with the addition of 1.5 ml of *hydrochloric acid,* complies with the limit test for *iron,* Appendix 2.3.4.

Chloride –1g, with the addition of 5 ml of *nitric acid,* complies with the limit test for *chlorides*, Appendix 2.3.2.

Sulphate –1 g, with the addition of 5 ml of *hydrochloric acid,* complies with the limit test for *sulphates,* Appendix 2.3.7.

Chromium –To 25 ml of a 2 per cent w/v solution in *water,* add about 0.2 g of *sodium peroxide and* boil gently for five minutes, cool, acidify with *dilute sulphuric acid* and add 2 drops of *diphenylcarbazide solution;* no violet colour is produced.

Assay –Weigh accurately about 3 g, dissolve in 50 ml of *water,* and titrate with *N hydrochloric acid,* using *bromophenol blue solution* as indicator. At the first colour change, boil the solution, cool, and complete the titration. Each ml of *N hydrochloric acid* is equivalent to 0.06911 g of K2CO_3.

Potassium Carbonate, Anhydrous. –Potassium carbonate dried at 135º for two hours spread in a thin layer and then cooled in a desiccator.

Potassium Chlorate – $KClO_3$ =122.55

Contains not less than 99.0 per cent of $KClO_3$.

Description –White powder or colourless crystals. In admixture with organic or readily oxidisable substances, it is liable to explode if heated or subjected to percussion or trituration.

Solubility –Soluble in *water*, and in *glycerin*; practically insoluble in *alcohol.*

Lead –Not more than 10 parts per million, Appendix 2.3.5.

Chloride –0.5 g complies with the limit test for *chlorides,* Appendix 2.3.2.

Sulphate –0.5 g complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 0.3 g and dissolve in 10 ml of *water* in a stoppered-flask, add 1 g of *sodium nitrate,* dissolved in 10 ml of *water,* and then 20 ml of *nitric acid;* stopper the flask and allow to stand for ten minutes; and 100 ml of water and sufficient *potassium permangnate solution* to produce a permanent pink colour; decolorise by the addition of a trace of *ferrous sulphate* and add 0.1 g of *urea.* Add 30 ml of 0.1 *N silver nitrate,* filter, wash with *water*, and titrate the filtrate and washings with 0.1 *N ammonium thiocyanate,* using *ferric ammonium sulphate solution* as indicator. Each ml of 0.1 *N silver nitrate* is equivalent to 0.01226 g of $KClO_3$

Potassium Chloride –KCl = 74.55

Analytical reagent grade

Potassium Chromate – K_2CrO_4 = 194.2

Analytical reagent grade

Potassium Chromate Solution –A 5.0 per cent w/v solution of potassium chromate.

Gives a red precipitate with *silver nitrate* in neutral solutions.

Potassium Cupri-Tartrate Solution –Cupric Tatrate Alkaline Solution : Fehling's Solution.

(1) **Copper Solution** – Dissolve 34.66 g of carefully selected small crystals of *copper sulphate,* showing no trace of efflorescence or of adhering moisture, in sufficient water to make 500 ml. Keep this solution in small, well-stoppered bottles.

(2) **Alkaline Tartrate Solution** – Dissolve 176 g of sodium *potassium tartrate* and 77 g of *sodium hydroxide* in sufficient *water* to produce 500 ml.

Mix equal volumes of the solutions No. 1 and No. 2 at the time of using.

Potassium Cyanide –KCN =65.12

Contains not less than 95.0 per cent of KCN.

Description – White, crystalline powder, gradually decomposing on exposure to air.

Solubility –Readily soluble in *water,* forming a clear, colourless solution.

Heavy metals – To 20 ml of a 5 per cent w/v solution in *water,* add 10 ml of *hydrogen sulphide solution;* no darkening is produced immediately or on the addition of 5 ml of *dilute hydrochloric acid.*

Assay – Weigh accurately about 0.5 g and dissolve in 50 ml of *water*, add 5 ml of dilute *ammonia solution* and 1 drop of *potassium iodide solution*; titrate with 0.1 *N silver nitrate until a faint* permanent turbidity appears. Each ml of 0.1 *N silver nitrate* is equivalent to 0.01302 g of KCN.

Potassium Cyanide Solution –A 10.0 per cent w/v solution of *potassium cyanide* in *water.*

Potassium Cyanide Solution, Lead –free –Weigh accurately about 10 g of *potassium cyanide* and dissolve in 90 ml of *water,* add 2 ml of *hydrogen peroxide solution,* allow to stand for twenty-four hours, and make up to 100 ml with *water*. It complies with the following tests.

Mix 2 ml with 5 ml of *lead-free ammonia solution* and 40 ml of water, and add 5 ml of *standard lead solution;* no darkening is produced.

Potassium Dichromate – $K_2Cr_2O_7$ =294.18.

Contains not less than 99.8 per cent of $K_2Cr_2O_7$

Description – Orange-red crystals or a crystalline powder.

Solubility – Soluble in *water*

Chloride. –To 20 ml of a 5 per cent w/v solution in *water* and 10 ml *nitric acid,* warm to about 50º and add a few drops of *silver nitrate solution*; not more than a faint opalescence is produced.

Assay –Carry out the Assay described under Potassium Chromate, using 2 g. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.004904 g of $K_2Cr_2O_7$.

Potassium Dichromate Solution – A 7.0 per cent w/v solution of *potassium dichromate* in *water.*

Potassium Dichromate, Solution 0.1N – $K_2Cr_2O_7$ = 294.18, 4.903 g in 1000 ml.

Weigh accurately 4.903 g of *potassium dichromate* and dissolve in sufficient *water* to produce 1000 ml.

Potassium Dihydrogen Phosphate - KH_2PO_4 = 136.1

Analytical reagent grade of commerce.

Potassium Ferricyanide – $K_3Fe(CN)_6$ = 329.25

Contains not less than 99.0 per cent of $K_3Fe(CN)_6$

Description – Ruby-red crystals.

Solubility – Very soluble in *water.*

Ferrocyanide – Rapidly wash 1 g with water, then dissolve in 100 ml of *water,* and add 1 drop of *ferric ammonium sulphate solution;* no blue colour is produced.

Assay – Weigh accurately about 1 g and dissolve in 50 ml of *water,* add 5 g of *potassium iodide* and 3 g of *zinc sulphate*, and titrate the liberated *iodine* with 0.1 *N sodium thiosulphate, using starch solution*, added towards the end of the titration, as indicator.Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.03293 g of $K_3Fe(CN)_6$.

Potassium Ferricyanide Solution – Wash about 1 g of *potassium ferricyanide* crystals with a little *water,* and dissolve the washed crystals in 100 ml of *water*.

Potassium Ferricyanide solution must be freshly prepared.

Potassium Ferrocyanide – $K_4Fe(CN)_6$, $3H_2O$ =422.39

Contains not less than 99.0 per cent of $K_4Fe(CN)_6$, $3H_2O$.

Description –Yellow, crystalline powder.

Solubility –Soluble in water.

Acidity or Alkalinity –A 10 per cent w/v solution in water is neutral to litmus paper.

Assay –Weigh accurately about 1g and dissolve in 200 ml of *water,* add 10 ml of *sulphuric acid and* titrate with 0.1 *N potassium permanganate.* Each ml of 0.1 *N potassium permanganate* is equivalent to 0.04224 g of $K_4Fe(CN)_6$, $3H_2O$.

Potassium Ferrocyanide Solution –A 5.0 per cent w/v solution of *potassium ferrocyanide in water.*

Potassium Hydrogen Phthalate –CO_2H. C_6H_4. CO_2K =204.22.

Contains not less than 99.9 per cent and not more than the equivalent of 100.1 per cent of $C_8H_5O_4K$ calculated with reference to the substance dried at 110° for one hour.

Description –White, crystalline powder.

Solubility –Slowly soluble in *water,* forming clear, colourless solution.

Acidity –A 2.0 per cent w/v solution in carbon dioxide free water gives with *bromophenol blue solution* the grey colour indicative of pH 4.0.

Assay –Weigh accurately about 9 g, dissolve in 100 ml of *water* and titrate with *N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *N Sodium hydroxide* is equivalent to 0.2042 g of $C_8H_5O_4K$.

Potassium Hydrogen Phthalate, 0.02 M –

Dissolve 4.084 g of *Potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydrogen Phthalate, 0.2 M – Dissolve 40.84 g of *potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydroxide –Caustic Potash : KOH = 56.11

Contains not less than 85.0 per cent of total alkali, calculated as KOH and not more than 4.0 per cent of K2CO3.

Description –Dry white sticks, pellets or fused mass; hard, brittle and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive.

Solubility –Freely soluble in water, in alcohol and in glycerin; very soluble in boiling ethyl alcohol.

Aluminium, iron and matter insoluble in hydrochloric acid –Boil 5 g with 40 ml of dilute hydrochloric acid, cool, make alkaline with dilute ammonia solution, boil, filter and wash the residue with a 2.5 per cent w/v solution of ammonium nitrate; the insoluble residue, after ignition to constant weight, weighs not more than 5 mg.

Chloride –0.5 g dissolved in water with the additon of 1.6 ml of nitric acid, complies with the limit test for chlorides, Appendix 2.3.2.

Heavy metals –Dissolve 1 g in a mixture of 5 ml of water and 7 ml of dilute hydrochloric acid. Heat to boiling, add 1 drop of phenolphthalein solution and dilute ammonia solution dropwise to produce a faint pink colour. Add 2 ml of acetic acid and water to make 25 ml; the limit of heavy metals is 30 parts per million, Appendix 2.3.3.

Sulphate –Dissolve 1 g in water with the addition of 4.5 ml of hydrochloric acid; the solution complies with the limit test for sulphates, Appendix 2.3.7.

Sodium –To 3 ml of a 10 per cent w/v solution add 1 ml of water, 1.5 ml of alcohol, and 3 ml of potassium antimonate solution and allow to stand; no white crystalline precipitate or sediment is visible to the naked eye within fifteen minutes.

Assay –Weigh accurately about 2 g, and dissolve in 25 ml of water, add 5 ml of barium chloride solution, and titrate with N hydrochloric acid, using phenolphthalein solution as indicator. To the solution in the flask add bromophenol blue solution, and continue the titration with N hydrochloric acid. Each ml of N hydrochloric acid, used in the second titration in equivalent to 0.06911 g of K2CO3 . Each ml of N hydrochloric acid, used in the combined titration is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage –Potassium Hydroxide should be kept in a well-closed container.

Potassium Hydroxide, xN –

Solution of any normality, x N, may be prepared by dissolving 56.11x g of potassium hydroxide in water and diluting to 1000 ml.

Potassium Hydroxide Solution –Solution of Potash.

An aqueous solution of potassium hydroxide containing 5.0 per cent w/v of total alkali, calculated as KOH (limits, 4.75 to 5.25).

Assay – Titrate 20 ml with N sulphuric acid, using solution of methyl orange as indicator. Each ml of N sulphuric acid is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage – Potassium hydroxide solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

Potassium Iodate – KIO3 = 214.0

Analytical reagent grade.

Potassium Iodate Solution – A 1.0 per cent w/v solution of potassium iodate in water.

Potassium Iodate, 0.05 M – KIO3 – 214.0; 10.70 g in 1000 ml

Weigh accurately 10.700 g of potassium iodate, previously dried at 110º to constant weight, in sufficeint water to produce 1000 ml.

Potassium Iodide –KI = 166.00

Description –Colourless crystals or white powder; odourless, taste, saline and slightly bitter.

Solubility –Very soluble in water and in glycerin; soluble in alcohol.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals -Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 2.3.3.

Barium –Dissolve 0.5 g in 10 ml of water and add 1 ml of dilute sulphuric acid; no turbidity develops within one minute.

Cyanides –Dissolve 0.5 g in 5 ml of warm water, add one drop of ferrous sulphate solution and 0.5 ml of sodium hydroxide solution and acidify with hydrochloric acid; no blue colour is produced.

Iodates –Dissolve 0.5 g in 10 ml of freshly boiled and cooled water, and add 2 drops of dilute sulphuric acid and a drop of starch solution; no blue colour is produced within two minutes.

Assay –Weigh accurately about 0.5 g, dissolve in about 10 ml of water and add 35 ml of hydrochloric acid and 5 ml of chloroform. Titrate with 0.05 M potassium iodate until the purple colour of iodine disappears from the chloroform. Add the last portion of the iodate solution drop-wise and agitate vigorously and continuously. Allow to stand for five minutes. If any colour develops in the chloroform layer continue the titration. Each ml of 0.05 M potassium iodate is equivalent to 0.0166 mg of KI.

Storage –Store in well-closed containers.

Potassium Iodide, M –Dissolve 166.00 g of potassium iodide in sufficient water to produce 1000 ml.

Potassium Iodide and Starch Solution –Dissolve 10 g of potassium iodide in sufficeint water to produce 95 ml and add 5 ml of starch solution.

Potassium Iodide and Starch solution must be recently prepared.

Potassium Iodide Solution –A 10 per cent w/v solution of potassium iodide in water.

Potassium Iodobismuthate Solution –Dissolve 100 g of tartaric acid in 400 ml of water and 8.5 g of bismuth oxynitrate. Shake during one hour, add 200 ml of a 40 per cent w/v solution of potassium iodide, and shake well. Allow to stand for twenty four hours and filter.

Potassium Iodobismuthate Solution, Dilute –Dissolve 100 g of tartaric acid in 500 ml of water and add 50 ml of potassium iodobismuthate solution.

Potassium Mercuric-Iodide Solution –Mayer's Reagent.

Add 1.36 g of mercuric chloride dissolved in 60 ml of water to a solution of 5 g of potassium iodide in 20 ml of water, mix and add sufficient water to produce 100 ml.

Potassium Mercuri-Iodide Solution, Alkaline (Nessler's Reagent)

To 3.5 g of potassium iodide add 1.25 g of mercuric chloride dissolved in 80 ml of water, add a cold saturated solution of mercuric chloride in water, with constant stirring until a slight red precipitate remains. Dissolve 12 g of sodium hydroxide in the solution, add a little more of the cold saturated solution of mercuric chloride and sufficient water to produce 100 ml. Allow to stand and decant the clear liquid.

Potassium Nitrate - KNO_3 = 101.1

Analytical reagent grade.

Potassium Permanganate – $KMnO_4$ = 158.03

Description –Dark purple, slender, prismatic crystals, having a metallic lustre, odourless; taste, sweet and astringent.

Solubility –Soluble in *water*; freely soluble in *boiling water.*

Chloride and Sulphate –Dissolve 1 g in 50 ml of boiling *water*, heat on a water-bath, and add gradually 4 ml or a sufficient quantity of *alcohol* until the meniscus is colour-less; filter. A 20 ml portion of the filtrate complies with the limit test for *chloride,* Appendix 2.3.2., and another 20 ml portion of the filtrate complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 0.8 g, dissolve in water and dilute to 250 ml. Titrate with this solution 25.0 ml of 0.1 *N oxalic acid* mixed with 25 ml of *water* and 5 ml of *sulphuric acid.* Keep the temperature at about 70º throughout the entire titration. Each ml of 0.1 *N oxalic acid* is equivalent to 0.00316 g of $KMnO_4$.

Storage –Store in well-closed containers.

Caution –Great care should be observed in handling *potassium permanganate*, as dangerous explosions are liable to occur if it is brought into contact with organic or other readily oxidisable substance, either in solution or in the dry condition.

Potassium Permanganate Solution – A 1.0 per cent w/v solution of *potassium permanganate* in water.

Potassium Permanganate, 0.1 N Solution –158.03. 3.161 g in 1000 ml

Dissolve about 3.3. g of *potassium permanganate* in 1000 ml of *water*, heat on a water-bath for one hour and allow to stand for two days. Filter through glass wool and standardise the solution as follows :

To an accurately measured volume of about 25 ml of the solution in a glass stoppered flask add 2 g of *potassium iodide* followed by 10 ml of *N sulphuric acid.* Titrate the liberated *iodine* with standardised 0.1 *N sodium thiosulphate,* adding 3 ml of *starch solution* as the end point is approached. Correct for a blank run on the same quantities of the same reagents. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.003161 g of $KMnO_4$

Potassium Tetraoxalate - $KH_3 (C_2O_4)_2, 2H_2O$ = 254.2.

Analytical reagent grade of commerce.

Potassium Thiocyanate –KCNS =97.18.

Analytical reagent grade.

Purified Water –H_2O = 18.02.

Description –Clear, colourless liquid, odourless, tasteless.

Purified water is prepareed from potable water by distillation, ion-exchange treatment, reverse osmosis or any other suitable process. It contains no added substances.

*p*H – Between 4.5 and 7.0 determined in a solution prepared by adding 0.3 ml of a saturated solution of *potassium chloride* to 100 ml of the liquid being examined.

Carbon dioxide –To 25 ml add 25 ml of *calcium hydroxide solution*, no turbidity is produced.

Chloride –To 10 ml add 1 ml of *dilute nitric acid* and 0.2 ml of *silver nitrate solution;* no opalescence is produced.

Sulphate –To 10 ml add 0.1 ml of *dilute hydrochloric acid* and 0.1 ml of *barium chloride solution :* the solution remains clear for an hour.

Nitrates and Nitrites –To 50 ml add 18 ml of *acetic acid* and 2 ml of *naphthylamine-sulphanilic acid* reagent. Add 0.12 g of *zinc reducing mixture* and shake several times. No pink colour develops within fifteen minutes.

Ammonium – To 20 ml add 1 ml of *alkaline potassium mercuric-iodide solution* and after five minutes view in a Nessler cylinder placed on a white tile; the colour is not more intense than that given on adding 1 ml of *alkaline potassium mercuric-iodide solution* to a solution containing 2.5 ml of *dilute ammonium chloride solution (Nessler's)* 7.5 ml of the liquid being examined.

Calcium –To 10 ml add 0.2 ml of *dilute ammonia solution* and 0.2 ml of *ammonium oxalate solution;* the solution remains clear for an hour.

Heavy metals –Adjust the pH of 40 ml to between 3.0 and 4.0 with *dilute acetic acid,* add 10 ml of freshly prepared *hydrogen sulphide solution* and allow to stand for ten minutes; the colour of the solution is not more than that of a mixture of 50 ml of the liquid being examined and the same amount of *dilute acetic acid* added to the sample.

Oxidisable matter –To 100 ml add 10 ml of *dilute sulphuric acid* and 0.1 ml of 0.1 *N potassium permanganate* and boil for five minutes. The solution remains faintly pink.

Total Solids –Not more than 0.001 per cent w/v determined on 100 ml by evaporating on a water bath and drying in an oven at 105º for one hour.

Storage –Store in tightly closed containers.

Resorcinol –Benzene –1,3 diol; $C_6H_4 (OH)_2$ = 110.1

Analytical reagent grade.

Colourless crystals or crystalline powder, melting point about 111º.

Resorcinol Solution –

Shake 0.2 g of *resorcinol* with 100 ml of toluene until saturated and decant.

Safranine – Basic red 2

Microscopical staining grade.

A reddish-brown powder.

Safranine Solution –

Saturated solution of *safranine* in *ethanol* (70 per cent.)

Sesame Oil –

Description – A pale yellow oil, odour, slight; taste, bland.

Solubility –Slightly soluble in alcohol; miscible with *chloroform,* with *solvent ether,* with *light petroleum* (b.p. 40º to 60º) and with *carbon disulphide.*

Refaractive index – At 40º, 1.4650 to 1.4665.

Wt. Per ml – At 25º, 0.916 to 0.921 g.

Storage –Preserve sesame oil in well-closed container protected from light, and avoid exposure to excessive heat.

Silver Carbonate – Ag_2CO_3 = 214

Prepared from *silver nitrate* and soluble *carbonate solution.* Light yellow powder when freshly precipitated, but becomes darker on drying and on exposure to light.

Silica Gel –

Partially dehydrated, polymerised, colloidal silicic acid containing cobalt chloride as an indicator.

Description –Blue granules, becoming pink when the moisture absorption capacity is exhausted. Silica Gel absorbs about 30 per cent of its weight of water at 20º. Its absorptive capacity may be regenerated by heating at 150º for two hours.

Silver Nitrate – $AgNO_3$ = 169.87

Description –Colourless crystals or white crystalline powder; odourless; taste, bitter and metallic.

Solubility –Very soluble in *water*, sparingly soluble in *alcohol;* slightly soluble in *solvent ether.*

Clarity and colour of solution –A solution of 2 g in 20 ml of water is clear and colourless.

Bismuth, Copper and Lead –To a solution of 1 g in 5 ml of *water, add a slight excess* of dilute *ammonia solution;* the mixute remains clear and colourless.

Foreign substances –To 30 ml of 4. 0 per cent w/v solution add 7.5 ml of 2 *N hydrochloric acid,* shake vigorously, filter and evaporate 10 ml of the filtrate to dryness on a water-bath; the residue weighs not more than 1 mg.

Assay – Weigh accurately about 0.5 g and dissolve in 50 ml of *water,* add 2 ml of *nitric acid,* and titrate with 0.1 *N ammonium thiocyanate,* using *ferric ammonium sulphate solution* as indicator. Each ml of 0.1 *N ammonium thiocyanate* is equivalent to 0.01699 g of $Ag\ NO_3$.

Storage –Store in tightly-closed, light resistant containers.

Silver Nitrate Solution –

A freshly prepared 5.0 per cent w/v solution of silver nitrate in water.

Silver Nitrate, 0.1 N– $Ag\ NO_3$ = 169. 87; 16.99 g in 1000 ml. Dissolve about 17 g in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.1 g of *sodium chloride* previously dried at 110º for two hours and dissolve in 5 ml of *water*. Add 5 ml of *acetic acid*, 50 ml of *methyl alcohol* and three drops of *eosin solution is* equivalent to 1 ml of 0.1 *N silver nitrate.*

Sodium Bicarbonate – $NaHCO_3$ =84.01

Description –White, crystalline powder or small, opaque, monoclinic crystals; odourless; taste, saline.

Solubility – Freely soluble in *water*; practically insoluble in *alcohol.*

Carbonate –pH of a freshly prepared 5.0 per cent w/v solution in *carbon dioxide-free water,* not more than 8.6.

Aluminium, calcium and insoluble matter –Boil 10 g with 50 ml of *water* and 20 ml of *dilute ammonia solution,* filter, and wash the residue with water; the residue, after ignition to constant weight, not more than 1 mg.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Iron –Dissolve 2.5 g in 20 ml of *water* and 4 ml of *iron-free hydrochloric acid,* and *dilute* to 40 ml with *water*; the solution complies with the *limit test for iron,* Appendix 2.3.4.

Heavy metals – Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner:

Mix 4.0 g with 5 ml of *water* and 10 ml of *dilute hydrochloric acid,* heat to boiling, and maintain the temperature for one minute. Add one drop of *phenolphthalein solution* and sufficient *ammonia solution* drop wise to give the solution a faint pink colour. Cool and dilute to 25 ml with *water,* Appendix 2.3.3.

Chlorides –Dissolve 1.0 g in *water* with the addition of 2 ml of *nitric acid;* the solution complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphates –Dissolve 2 g in *water* with the addition of 2 ml of *hydrochloric acid;* the solution complies with the limit test for *sulphates,* Appendix 2.3.7.

Ammonium compounds –1 g warmed with 10 ml of *sodium hydroxide solution* does not evolve ammonia.

Assay –Weigh accurately about 1 g, dissolve in 20 ml of *water,* and titrate with 0.5 *N sulphuric acid* using *methyl orange solution* as indicator. Each ml of 0.5 *N sulphuric acid* is equivalent to 0.042 g of $NaHCO_3$.

Storage –Store in well-closed containers.

Sodium Bicarbonate Solution –A 5 per cnet w/v solution of *sodium bicarbonate* in *water.*

Sodium Bisulphite – Consists of sodium bisulphite ($NaHSO_3$) and sodium metabisulphite ($Na2S2O_3$) in varying proportions. It yields not less than 58.5 per cent and not more than 67.4 per cent of SO_2.

Description –White or yellowish-white crystals or granular powder; odour of sulphur dioxide. It is unstable in air.

Solubility –Freely soluble in *water,* slightly soluble in *alcohol.*

Assay –Weigh accurately about 0.2 g and transfer to a glass-stoppered flask, add 50 ml of 0.1 *N iodine* and insert the stopper of the flask. Allow to stand for five minutes, add 1 ml of *hydrochloric acid,* and titrate the excess of iodine with 0.1 *N sodium thiosulphate,* using *starch solution* as indicator added towards the end of the titration. Each ml of 0.1 *N iodine* is equivalent to 0.003203 g of SO_2.

Storage – Preserve Sodium Bisulphite in tightly-closed containers in a cool place.

Sodium Bisulphite Solution – Dissolve 10 g of *sodium bisulphite* in sufficient *water* to make 30 ml.

Sodium Bisulphite Solution must be freshly prepared.

Sodium Carbonate – Na_2CO_3. $10H_2O$ =286.2.

Analytical reagent grade.

Sodium Chloride – NaCl = 58.44

Analytical reagent grade.

Sodium Cobaltinitrite – $Na_3CO(NO_2)_6$ = 403.94

Description – An orange-yellow powder.

Solubility – Readily soluble in *water,* forming a clear orange-red solution.

Potassium – Dissolve 3 g in 10 ml of *water*, add the solution to a mixture of 5 ml of *water* and 2 ml of *dilute acetic acid,* and allow to stand for one hour; no precipitate is produced.

Sodium Cobaltinitrite Solution – A 30 per cent w/v solution of *sodium cobaltinitrite* in *water.*

Sodium Diethyldithiocarbamate –$(C_2H_5)_2$, N. CS.SNa, $3H_2O$ = 225.30.

Description –White or colourless crystals.

Solubility – Readily soluble in *water*, yielding a colourless solution.

Sensitivity – Add 10 ml of a 0.1 per cent w/v solution to 50 ml of *water* containing 0.002 mg of copper previously made alkaline with *dilute ammonia solution.* A yellowish-brown colour should be apparent in the solution when compared with a blank test containing no copper.

Sodium Diethyldithiocarbamate Solution – A 0.1 per cent w/v solution of *sodium diethyldithiocarbamate* in *water.*

Sodium Hydroxide – NaOH = 40.00

Description – White sticks, pellets, fused masses, or scales; dry, hard brittle and showing a crystalline fracture, very deliquescent; strongly alkaline and corrosive.

Solubility – Freely soluble in *water* and in *alcohol.*

Aluminium, iron and matter insoluble in hydrochloric acid –Boil 5 g with 50 ml of dilute hydrochloric acid, cool, make alkaline with *dilute ammonia solution,* boil, filter, and wash with a 2.5 per cent w/v solution of *ammonium nitrate;* the insoluble residue after ignition to constant weight weighs not more than 5 mg.

Arsenic – Not more than 4 parts per million, Appendix 2.3.1.

Heavy metals – Not more than 30 parts per million, determined by Method A, Appendix 2.3.3. on a solution prepared by dissolving 0.67 g in 5 ml of water and 7 ml of 3 *N hydrochloric acid.* Heat to boiling, cool and dilute to 25 ml with water.

Potassium – Acidify 5 ml of a 5 per cent w/v solution with *acetic acid* and add 3 drops of *sodium cobaltnitrite solution;* no preciptitate is formed.

Chloride – 0.5 g dissolved in *water* with the addition of 1.8 ml of *nitric acid,* complies with the limit test for *chlorides,* Appendix 2.3.2.

Sulphates – 1 g dissolved in *water* with the addition of 3.5 ml of *hydrochloric acid* complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay – Weigh accurately about 1.5 g and dissolve in about 40 ml of *carbon dioxide-free water.* Cool and titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. When the pink colour of the solution is discharged, record the volume of acid solution required, add *methyl orange solution* and continue the titration until a persistent pink colour is produced. Each ml of *N sulphuric acid* is equivalent to 0.040 g of total alkali calculated as NaOH and each ml of acid consumed in the titration with *methyl orange* is equivalent to 0.106 g of Na_2CO_3.

Storage –Store in tightly closed containers.

Sodium Hydroxide, xN – Solutions of any normality, xN may be prepared by dissolving 40 x g of *sodium hydroxide* in *water* and diluting to 1000 ml.

Sodium Hydroxide Solution – A 20.0 per cent w/v solution of *sodium hydroxide* in *water.*

Sodium Hydroxide Solution, Dilute –

A 5.0 per cent w/v solution of *sodium hydroxide* in water.

Sodium Nitrite –$NaNO_2$ = 69.00, Analytical reagent grade.

Sodium Nitroprusside –(Sodium penta cyano nitrosyl ferrate (iii) dihydrate; $Na_2[Fe(CN)_5\ (NO)],\ 2H_2O$ = 298.0

Analytical reagent grade of commerce.

Sodium Peroxide – Na_2O_2 =77.98.

Analytical grade reagent.

Sodium Potassium Tartrate –Rochelle Salt COONa.CH(OH). CH(OH), COOK, $4H_2O$ = 282.17

Contains not less than 99.0 per cent and not more than the equivalent of 104.0 per cent of $C_4H_4O_6KNa$, $4H_2O$.

Description –Colourless crystals or a white, crystalline powder; odourless; taste saline and cooling. It effloresces slightly in warm, dry air, the crystals are often coated with a white powder.

Solubility – Soluble in *water;* practically insoluble in alcohol.

Acidity or Alkalinity – Dissolve 1 g in 10 ml of recently boiled and cooled *water,* the solution requires for neutralisation not more than 0.1 ml of 0.1 *N sodium hydroxide* or of 0.1 *N hydrochloric acid,* using *phenolphthalein solution as indicator.*

Iron – 0.5 g complies with the *limit test for iron,* Appendix 2.3.4.

Chloride – 0.5 g complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphate – 0.5 g complies with the *limit test for sulphate ,* Appendix 2.3.7.

Assay – Weigh accurately about 2 g and heat until carbonised, cool, and boil the residue with 50 ml of *water* and 50 ml of 0.5 *N sulphuric acid;* filter, and wash the filter with *water;* titrate the excess of acid in the filtrate and washings with 0.5 *N sodium hydroxide,* using *methyl orange solution* as indicator. Each ml of 0.5 *N sulphuric acid* is equivalent to 0.07056 g of $C_4H_4O_6KNa, 4H_2O$.

Sodium Sulphide –Na_2S + aq.

Analytical reagent grade. Deliquescent, crystalline masses turning yellow on storage.

Sodium Sulphide Solution –Dissolve with heating, 12 g of *sodium sulphide* in a mixture of 10 ml of *water* and 25 ml of *glycerol,* cool and dilute to 100 ml with the same mixture.

Sodium Sulphite, Anhydrous –Na_2SO_3 =126.06

Description –Small crystals or powder.

Solubility –Freely soluble in *water,* soluble in *glycerin;* almost insoluble in *alcohol.*

Sodium Thiosulphate – $Na_2S_2O_3$, 5H2O =248.17.

Description – Large colourless crystals or coarse, crystalline powder; odourless; taste, saline, deliquescent in moist air and effloresces in dry air at temperature above 33º.

Solubility – Very soluble in *water;* insoluble in *alcohol.*

pH – Between 6.0 and 8.4, determined in a 10 per cent w/v solution.

Arsenic – Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals – Not more than 20 parts per million, determined by Method A, Appendix 2.3.3. on a solution prepared in the following manner : Dissolve 1 g in 10 ml of *water,* slowly add 5 ml of *dilute hydrochloric acid* and evaporate the mixture to dryness on a water-bath. Gently boil the residue with 15 ml of *water* for two minutes, and filter. Heat the filtrate to boiling, and add *sufficient bromine solution* to the hot filtrate to produce a clear solution and add a slight excess of *bromine solution.* Boil the solution to expel the *bromine* completely, cool to room temperature, then add a drop of *phenolphthalein solution* and *sodium hydroxide solution* until a slight pink colour is produced. Add 2 ml of *dilute acetic acid* and dilute with *water* to 25 ml.

Calcium –Dissolve 1 g in 20 ml of *water,* and add a few ml of *ammonium oxalate solution;* no turbidity is produced.

Chloride –Dissolve 0.25 g in 15 ml of *2N nitric acid* and boil gently for three to four minutes, cool and filter; the filtrate complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphate and Sulphite –Dissolve 0.25 g in 10 ml of *water*, to 3 ml of this solution add 2 ml of *iodine solution,* and gradually add more *iodine solution*, dropwise until a very faint-persistant yellow colour is procduced; the resulting solution complies with the limit test for sulphates, Appendix 2.3.7.

Sulphide –Dissolve 1 g in 10 ml of *water* and 10.00 ml of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside;* the solution does not become violet.

Assay –Weigh accurately about 0.8 g and dissolve in 30 ml of *water.* Titrate with 0.1 *N iodine,* using 3 ml of *starch solution* as indicator as the end-point is approached. Each ml of 0.1 iodine is equivalent to 0.02482 g of $Na_2S_2O_3$, $5H_2O$.

Storage – Store in tightly-closed containers.

Sodium Thiosulphate 0.1 N – $Na_2S_2O_3$, $5H_2O$. = 248.17, 24.82 g in 1000 ml.

Dissolve about 26 g of *sodium thiosulphate* and 0.2 g of *sodium carbonate* in *carbon dioxide-free water* and dilute to 1000 ml with the same solvent. Standardise the solution as follows :

Dissolve 0.300 g of *potassium bromate* in sufficient *water* to produce 250 ml. To 50 ml of this solution, add 2 g of *potassium iodide* and 3 ml of 2 *N hydrochloric acid* and titrate with the *sodium-thiosulphate solution* using *starch solution,* added towards the end of the titration, as indicator until the blue colour is discharged. Each 0.002784 g of *potassium bromate* is equivalent to 1 ml of 0.1*N sodium thiosulphate.* Note: –Re-standardise 0.1 *N sodium thiosulphate* frequently*.*

Stannous Chloride – $SnCl_2$, $2H_2O$ =225.63.

Contains not less than 97.0 per cent of $SnCl_2$, $2H_2O$.

Description –Colourless crystals.

Solubility – Soluble in *dilute hydrochloric acid.*

Arsenic - Dissolve 5.0 g in 10 ml of *hydrochloric acid,* heat to boiling and allow to stand for one hour; the solution shows no darkening when compared with a freshly prepared solution of 5.0 g in 10 ml of *hydrochloric acid.*

Sulphate – 5.0 g with the addition of 2 ml of *dilute hydrochloric acid*, complies with the *limit test for sulphates,* Appendix 2.3.7.

Assay – Weigh accurately about 1.0 g and dissolve in 30 ml of *hydrochloric acid* in a stoppered flask. Add 20 ml of *water* and 5 ml of *chloroform* and titrate rapidly with 0.05 *M potassium iodate until* the *chloroform* layer is colourless. Each ml of 0.05 M potassium iodate is equivalent to 0.02256 g of $SnCl_2$, $2H_2O$.

Stannous Chloride Solution – May be prepared by either of the two methods given below :

Dissolve 330 g of stannous *chloride* in 100 ml of *hydrochloric acid* and add sufficient *water* to produce 1000 ml.

Dilute 60 ml of *hydrochloric acid* with 20 ml of *water*, add 20 g of tin and heat gently until gas ceases to be evolved; add sufficient *water* to produce 100 ml, allowing the undissolved tin to remain in the solution.

Starch Soluble – Starch, which has been treated with *hydrochloric acid* until after being washed, it forms an almost clear liquid solution in hot water.

Description – Fine, white powder.

Solubility – Soluble in hot *water,* usually forming a slightly turbid *solution.*

Acidity or Alkalinity – Shake 2 g with 20 ml of *water* for three minutes and filter; the filtrate is not alkaline or more than fainthy acid to litmus paper.

Sensitivity – Mix 1 g with a little cold *water* and add 200 ml *boiling water.* Add 5 ml of this solution to 100 ml of *water* and add 0.05 ml of 0.1 *N iodine.* The deep blue colour is discharged by 0.05 ml of 0.1 *N sodium thiosulphate.*

Ash – Not more than 0.3 per cent, Appendix 2.2.3.

Starch Solution – Triturate 0.5 g of *soluble starch,* with 5 ml of *water,* and add this, with constant stirring, to sufficient water to produce about 100 ml. Boil for a few minutes, cool, and filter.

Solution of *starch must be recently prepared.*

Sudan Red G – Sudan III; Solvent Red 23; 1-(4-Phenyl-azophenylazo)-2-naphthol; $C_{22}H_{16}N_4O$ = 352.40.

Description – Reddish-brown powder.

Solubility – Insoluble in *water*; soluble in *chloroform,* in *glacial acetic acid;* moderately soluble in *alcohol,* in solvent *ether* and in *acetone*.

Sulphamic Acid – NH_2SO_3H =97.09.

Contains not less than 98.0 per cent of H_3NO_3S.

Description - White crystals or a white crystalline powder.

Solubility – Readily soluble in water.

Melting Range – 203° to 205°, with decomposition.

Sulphuric Acid – H2SO4 = 98.08.

When no molarity is indicated use analytical reagent grade of commerce containing about 98 per cent w/w of *sulphuric acid.* An oily, corrosive liquid weighing about 1.84 g per ml and about 18 M in strength.

When solutions of molarity xM are required, they should be prepared by carefully adding 54 ml of sulphuric acid to an equal volume of water and diluting with water to 1000 ml.

Solutions of sulphuric acid contain about 10 per cent w/v of H_2SO_4 per g mol.

Sulphuric Acid, Dilute – Contains approximately 10 per cent w/w of H_2SO_4.

Dilute 57 ml of sulphuric acid to 1000 ml with water.

Sulphuric Acid, Chlorine-free – Sulphuric acid which complies with the following additional test:

Chloride – Mix 2 ml with 50 ml of water and add 1 ml of solution of *silver nitrate,* no opalescence is produced.

Sulphuric Acid, Nitrogen-free-Sulphuric acid which contains not less than 98.0 per cent w/w of H_2SO_4 and complies with the following additional test :

Nitrate – Mix 45 ml with 5 ml of *water*, cool and add 8 mg of *diphenyl benezidine;* the solution is colourless or not more than very pale blue.

Tartaric Acid – $(CHOH. COOH)_2$ =150.1

Analytical reagent grade.

Thioglycollic Acid – Mercapto acetic acid, – HS. CH_2COOH =92.11.

Contains not less than 89.0 per cent w/w of $C_2H_4O_2S$, as determined by both parts of the Assay described below :

Description –Colourless or nearly colourless liquid; odour strong and upleasant.

Iron –Mix 0.1 ml with 50 ml of water and render alkaline with *strong ammonia solution;* no pink colour is produced.

Assay – Weigh accurately about 0.4 g and dissolve in 20 ml of *water* and titrate with 0.1 *N sodium hydroxide* using *cresol red solution* as indicator. Each ml of 0.1 *N sodium hydroxide is equivalent to* 0.009212 g of $C_2H_4O_2S$.

To the above neutralised solution and 2 g of *sodium bicarbonate* and titrate with 0.1 *N iodine* Each ml of 0.1 *N iodine is equivalent to 0.009212 g of* $C_2H_4O_2S$.

Thymol – 2-Isopropyl-5-methylphenol; $C_{10}H_{14}O$ = 150.2

General reagent grade.

Colourless crystals with an aromatic odour; freezing point not below 49º.

Thymol Blue –6, 6′ –(3H-2, 1 Benzoxathil –3 –ylidene) dithymol SS =dioxide; $C_{27}H_{30}O_5S$ = 466.6

Gives a red colour in strongly acid solutions, a yellow colour in weakly acid and weakly alkaline solutions, and a blue colour in more strongly alkaline solutions (pH range, 1.2 to 2.8 and 2.0 to 9.6).

Thymol Blue Solution – Warm 0.1 g of *thymol blue* with 4.3 ml of 0.05 M sodium hydroxide and 5 ml of *ethanol (90 per cent);* after solution is effected add sufficient *ethanol (20 per cent)* to produce 250 ml.

Complies with the following test –

Sensitivity – A mixture of 0.1 ml and 100 ml of carbon dioxide-free water to which 0.2 ml of 0.02 *N sodium hydroxide* has been added is blue. Not more than 0.1 ml of 0.2 *N hydrochloric* acid is required to change the colour to yellow.

Titanous Chloride Solution – General reagent grade of commerce containing about 15 per cent w/v to TiCl3.

Weight per ml, about 1.2 g.

Dull purplish liquid with a strongly acid reaction.

Titanous Chloride 0.1 N – $TiCl_3$=154.26; 15.43 g in 1000 ml.

Add 103 ml of *titanous chloride solution* to 100 ml of *hydrochloric acid,* dilute to 1000 ml with recently boiled and cooled water, and mix, standardise, immediately before use, as follows :

Place an accurately measured volume of about 30 ml of standardised 0.1 *N ferric ammonium sulphate* in a flask and pass in a rapid stream of *carbon dioxide* until all the air has been removed. Add the *titanous chloride solution* from a burette and in an atmosphere of carbon dioxide until near the calculated end point then add 5 ml of *ammonium thiocyanate solution,* and continue the titration until the solution is colourless. Each ml of 0.1 *N ferric ammonium sulphate* is equivalent to 0.01543 g of $TiCl_3$.

Vanillin-Sulphuric Acid Reagent – 5 % Ethanolic sulphuric acid (Solution I)

1 % Ethanolic vanillin (Solution II)

The plate is sprayed vigorously with 10 ml Solution I, followed immediately by 5-10 ml of Solution II.

Water –See purified water.

Water, Ammonia-free –Water, which has been boiled vigorously for a few minutes and protected from the atomosphere during cooling and storage.

Xylenol Orange – [3H-2,1-Benzoxathiol–3-ylidene bis – (6-hydroxy-5-methyl-m-phenylene) methylenenitrilo] tetra acetic acid SS-dioxide or its tetra sodium salt.

Gives a reddish-purple colour with mercury, lead, zinc and contain other metal ions in acid solution. When metal ions are absent, for example, in the presence of an excess of *disodium ethylenediamine tetraacetate*, this solution is yellow.

Xylenol Orange Solution –Shake 0.1 g of *xylenol orange* with 100 ml of *water* and filter, if necessary.

Zinc, Granulated –Zn=65.38.

Analytical reagent grade of commerce.

Zinc Powder –Zn =65.38.

Analytical reagent grade of commerce.

Zinc Sulphate –$ZnSO_4$, $7H_2O$ = 287.6.

Analytical reagent grade of commerce.